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REVIEW

Non-coding RNAs: Role in diabetic foot and wound healing

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Abstract

Diabetic foot ulcer (DFU) and poor wound healing are chronic complications in patients with diabetes. The increasing incidence of DFU has resulted in huge pressure worldwide. Diagnosing and treating this condition are therefore of great importance to control morbidity and improve prognosis. Finding new markers with potential diagnostic and therapeutic utility in DFU has gathered increasing interest. Wound healing is a process divided into three stages: Inflammation, proliferation, and regeneration. Non-coding RNAs (ncRNAs), which are small protected molecules transcribed from the genome without protein translation function, have emerged as important regulators of diabetes complications. The deregulation of ncRNAs may be linked to accelerated DFU development and delayed wound healing. Moreover, ncRNAs can be used for therapeutic purposes in diabetic wound healing. Herein, we summarize the role of microRNAs, long ncRNAs, and circular RNAs in diverse stages of DFU wound healing and their potential use as novel therapeutic targets.

Key Words: Diabetic foot ulcer; Wound healing; MicroRNA; Long non-coding RNAs; Circular RNAs; Inflammation; Proliferation; Regeneration

Core Tip: Non-coding RNAs (ncRNAs) have emerged as important regulators of diabetic foot and wound healing. NcRNAs can be used for therapeutic purposes in diabetic wound healing. In this study, we summarize the roles of microRNAs, long ncRNAs, and circular RNAs in diverse stages of diabetic foot ulcer wound healing and their potential use as novel therapeutic targets.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease that is rapidly increasing worldwide. DM is a global public health burden with a negative impact on global health and socioeconomic development. Chronic hyperglycemia causes blood vessel inflammation, which leads to macroangiopathy and microangiopathy, particularly diabetic foot ulcer (DFU) and delayed wound healing. Delayed healing of chronic ulcer wounds in patients with diabetes is due to neuropathy, microangiopathy, and immune system dysfunction[1,2]. One of the leading causes of death in patients with diabetes is lower extremity amputation, which accounts for approximately 15% of DFU cases[3]. Different functional and structural microvascular changes in patients with diabetes increase the vulnerability of the skin and contribute to impaired wound healing[4]. DFU contributes to physical and psychological problems that hinder the health economy immensely. Conventional DFU treatments have an inefficient impact on reduction of the amputation rate; thus, a more efficient treatment is needed. Therefore, a better understanding of the molecular mechanisms and biomolecules involved in DFU development is necessary to provide better therapeutic options for wound healing.

Non-coding RNAs (ncRNAs) are potential novel biomarkers transcribed from the genome without protein translation function but can still perform specific biological functions. NcRNAs can be divided into two categories depending on the length of nucleotides; short-stranded RNAs or microRNAs (miRNAs) which are less than 200 nucleotides in length, and long ncRNAs (lncRNAs) which are greater than 200 nucleotides in length. Emerging evidence suggests that ncRNAs have an important regulatory role in various metabolic diseases, such as DM, based on the development of microarray and high-throughput sequencing[5]. In addition, some lncRNAs are covalently bound to the 3′-5′ end, forming circular RNAs (circRNAs)[6]. NcRNAs can be protected from the effects of RNA enzyme activity, temperature changes, and extreme pH values by binding to proteins or being packaged into extracellular vesicles. In this way, ncRNAs can maintain a stable state in the extracellular environment and can be used as a potential biomarker for diagnosing and treating diseases[7-9]. NcRNAs regulate cellular chromatin rearrangements, histone modifications, variable splicing gene modifications, or gene expression; mediate different biological processes; and ultimately influence the development of certain diseases[10]. Exosome-cargoed ncRNAs have been reported as pivotal regulators of angiogenesis during wound closure[11]. This background confers the possible treatment of delayed wound healing using ncRNAs. In this study, we summarize the role and mechanism of miRNAs, lncRNAs, and circRNAs in the pathogenesis and process of wound healing in DFU and the research progress of ncRNAs in cell therapy.

WOUND HEALING PROCESS

Wound healing is a complex and highly regulated process divided into three phases: Inflammation, proliferation, and regeneration[12]. Diabetic wound healing is widely associated with different cellular components and the extracellular matrix (ECM) in different parts of the skin[13]. The main effector cells in the inflammatory phase are macrophages. When normal skin is damaged, macrophages polarize to M1 phenotype, producing pro-inflammatory cytokines and stimulating endothelial cells and fibroblasts to release reactive oxygen species (ROS) to remove bacteria and debris from wounds. The subsequent shift to the M2 phenotype is correlated with remission of the inflammatory response and wound remodeling[14,15]. In diabetic wounds, the persistence of the M1 phenotype and the inability to subsequently polarize to the M2 phenotype are the key components delaying wound healing. Angiogenesis is the main basis of the proliferative phase of wound healing, cell proliferation, migration, and differentiation[14]. The integrity of the endothelial cell structure plays a very important role in maintaining normal blood circulation in the body. In healthy tissues, endothelial cells are in a quiescent phase. In diabetic patients, wound healing is slowed by decreased angiogenic growth factors, such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and hypoxia-inducible factor (HIF)-1α[16-18]. An unfavorable diabetic wound environment promotes the dysregulation of key signaling pathways, such as Notch and PI3K/AKT/eNOS[19,20]. The regenerative phase of wound healing includes re-epithelialization and ECM remodeling. Reduced blood flow restricts the migration of leukocytes, keratinocytes, fibroblasts, and endothelial cells to the wound, which is detrimental to wound healing[21]. Fibroblasts proliferate and secrete ECM components, such as collagen fibers, which provide supportive structures for cell proliferation and migration to restore skin tissue function and

integrity to maintain tissue elasticity and strength[22]. DFUs have collagen degeneration and deformation and reduced fibroblasts in the proliferation and migration stages[23]. Keratinocytes are the main constituent cells of the epidermis involved in skin wound healing through migration, proliferation, and differentiation[24]. In addition, epithelial-to-mesenchymal transition (EMT) plays a crucial role in DFU regeneration and wound healing[25]. Many studies have shown that ncRNAs regulate EMT involved in DFU and wound healing[26,27]. The wound healing process is shown in Figure 1.

MIRNAS

MiRNAs are a class of endogenous small ncRNAs with a molecular length of 18–25 nucleotides that regulate gene and/or protein expression at the post-transcriptional level by specifically binding to the 3′-untranslated region of downstream target miRNAs. The increased prevalence of diabetes has prompted increasing research into the mechanisms of miRNAs as therapeutic targets in DFU and wound healing. A study showed that low miR-24 expression is an independent risk factor for DFU in multifactorial logistic regression analysis[28]. Furthermore, low miR-24 expression is negatively correlated with fasting blood glucose and glycated hemoglobin and positively correlated with inflammatory markers[28-30]. MiRNAs have been associated with DFU progression and severity; specific miRNAs, such as miR-26, increase DFU severity[31], whereas other miRNAs, such as miR-129 and miR-335, improve wound healing[26].

Inflammation

MiR-217 belongs to the group that increases DFU severity. A study showed that a dual luciferase reporter gene assay confirmed HIF-1α as a direct target gene of miR-217. MiR-217 expression was upregulated whereas HIF-1α/VEGF expression was downregulated in patients with DFU and in the serum of rats with DFU compared with DM and healthy controls[32]. MiR-23c is upregulated in the peripheral blood and wound tissue in DFU, targeting stromal cell-derived factor-1α and inhibiting wound angiogenesis by recruiting inflammatory cells, such as macrophages[33]. In a mouse DFU model, miR-497 expression was downregulated, which considerably increased the expression of pro-inflammatory factors, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, resulting in a prolonged inflammatory phase of wound healing[34]. MiR-155 regulates insulin sensitivity and blood glucose levels in mice[35]. MiR-155 is markedly upregulated in diabetic skin[36]. MiR-155 has pro-inflammatory effects; thus, miR-155 inhibition leads to reduced inflammation, increased macrophage M2 polarization, reduced IL-1β and TNF-α levels, more regular collagen fiber alignment, and faster diabetic wound healing[37-39]. MiR-217, miR-497, and miR-155 are effector molecules in the inflammatory phase of diabetic wound healing; however, a further exploration of their mechanisms might improve wound healing during the inflammatory phase.

Proliferation

Angiogenesis is an essential step in the proliferative phase associated with DFU prognosis and wound healing. Recent studies have focused on the mechanisms and applications of miRNAs in regulating angiogenesis during the proliferative phase[40-42]. A maggot therapeutic approach study found that miR-18a/19a is markedly upregulated and thrombospondin-1 (TSP-1) expression is downregulated in DFU wounds as a result of impaired angiogenesis. The target activation of miR-18a/19a transcript levels and the regulation of TSP-1 expression may be a novel strategy for DFU treatment[40]. MiR-15a-3p is upregulated in the blood exosomes of patients with diabetes[41]. *In vivo* and *in vitro* experiments showed that exosomes with low miR-15a-3p expression inhibited diabetic wound healing. By contrast, knockdown of circulating exosomal miR-15a-3p expression may accelerate wound healing through the activation of NADPH oxidase (NOX) 5 and increase ROS release[41]. NOX activates redox signaling pathways and promotes angiogenesis[43]. Phosphatase and tensin homolog (PTEN) expression is regulated by blood glucose concentrations, is mainly found in epithelial cells, and activates signaling cascades that affect angiogenesis[44]. MiR-152-3p is an upstream negative regulator of PTEN upregulated in diabetic wounds; hence, inhibiting the angiogenic function of PTEN leads to delayed wound healing[45]. MiR-195-5p and miR-205-5p carried by extracellular vesicles in DFU wound fluid negatively regulate angiogenesis and wound healing in DFU[42]. Increased miR-133b expression induces downregulation of EGF receptor (EGFR), affecting endothelial cell proliferation and angiogenesis in all diabetic wounds. *In vitro* experiments showed that miR-133b downregulation in human umbilical vein endothelial cells partially reverses impaired angiogenesis[46]. These findings imply that miR-133b negatively regulates angiogenesis during the proliferative phase of wound healing. Huang *et al*[47] found that miR-489-3p downregulation increases sirtuin (SIRT) 1 expression, promotes the PI3K/AKT/eNOS signaling pathway, improves cellular antioxidant capacity, and alleviates DFU. MiR-199a-5p has an important role in the development of diabetes and its complications[48,49]. Moreover, miR-199a-5p promotes apoptosis and ROS production within pancreatic β-cells in type 2 DM (T2DM)[50]. MiR-199a-5p sponge-adsorbed to hsa-circ-006040 inhibits macrophage-mediated inflammatory responses in type 1 DM (T1DM)[48]. Wang *et al*[49] found that downregulating miR-199a-3p in

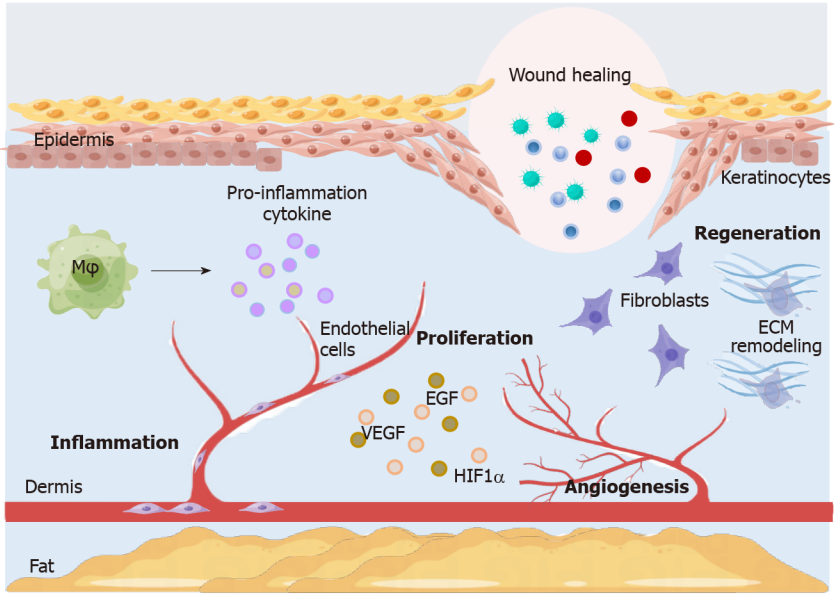

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Figure 1 A diagram of the diabetic foot wound healing process. In the inflammation phase, macrophages produce pro-inflammatory cytokines. In the proliferation phase, angiogenic growth factors promote angiogenesis by stimulating endothelial cell proliferation and migration. In the regeneration phase, fibroblasts proliferate and secrete extracellular matrix components to provide supportive structures for cell proliferation and migration to restore skin tissue function. Mφ: Macrophages; VEGF: Vascular endothelial growth factor; EGF: Epidermal growth factor; HIF-1α: Hypoxia-inducible factor 1 α; ECM: Extracellular matrix.

endothelial cells alleviates inhibition of the target VEGFA and Rho-related kinase 1, rescuing the cellular damage induced by high glucose and restoring angiogenic function. Therefore, these findings suggest that regulating miRNA expression during the proliferative phase of wound healing has great potential in DFU treatment and wound repair.

Regeneration

Recently, Moura *et al*[36] also found that the local inhibition of miR-155 in diabetic wounds increased the expression of its target, fibroblast growth factor (FGF) 7, which sequentially increased re-epithelialization and accelerated wound healing[36,51]. Yuan *et al*[52] found that miR-203 upregulation in DFU tissues may inhibit the EMT process and delay wound healing in a rat DFU model. On the contrary, miR-203 knockdown promoted wound healing by activating the target gene, IL-8, and IL-8/AKT downstream pathways. High miR-203 expression reduces keratinocyte proliferation and migration, partially explaining the development of DFU into chronic refractory wounds[52]. On the contrary, recent studies have found that negative pressure wound therapy can reverse the inhibition of keratinocytes as a result of high levels of miR-203 by reducing miR-203 in the peripheral blood and wound tissue and upregulating p63 expression[53]. Sprouty homolog (SPRY) 1, an antagonist of the FGF pathway, is expressed in fibroblasts, and its downregulation plays an important role in wound healing[54,55]. MiR-21-3p is downregulated in diabetic patients compared with healthy controls and in fibroblasts stimulated with D-glucose compared with control fibroblasts[56]. Enhanced miR-21-3p expression may inhibit SPRY1, stimulate fibroblast proliferation and migration, and accelerate wound healing[42]. MiR-146a is downregulated in DFU wound tissue. Bioinformatics analysis revealed that A-kinase-anchoring protein 12 (AKAP12) and Toll-like receptor 4 (TLR4) are the target genes of miR-146a. Peng *et al*[57] showed that miR-146a activates in the inflammatory phase of diabetic wound healing by inhibiting the TLR4/nuclear factor-kappaB axis involved in macrophage M2 polarization. In addition, Zhang *et al*[58] constructed an *in vitro* DFU model using human keratinocyte-derived HaCaT cells and demonstrated that miR-146a is activated during the tissue regeneration phase. *In vivo* and *ex vivo* results showed that miR-146a overexpression inhibited the angiogenic regulator AKAP12, activated the HIF-1α/Wnt3α/β-catenin signaling pathway, and promoted cell proliferation and migration[57]. MiRNAs have regulatory effects on a wide range of cells involved in tissue remodeling during the regeneration phase. MiRNAs are the most studied ncRNAs and act in various periods of DFU and wound healing, respectively, or continuously. We summarized some of the considerably altered miRNAs in diabetic patients as shown in Table 1. Notably, most of these pooled miRNAs have not been reported to have a clear therapeutic role in DFU and should therefore be evaluated in future studies.

Table 1 Micro-RNAs in diabetic foot and wound healing

Name	Expression	Animal	Target gene	Pathway	Phase	Ref.
miRNA-217	Up	Mouse	*HIF-1α*	VEGF	Inflammation	Lin *et al*[32], 2019
miRNA-23c	Up	/	*SDF-1α*	SDF-1α/CXCL12	Inflammation	Amin *et al*[33], 2020
miRNA-497	Down	Mouse	*IL-1β, IL-6, TNF-α*	NF-κB	Inflammation	Ban *et al*[34], 2020
miRNA-155	Up	Mouse	*FGF7*	/	Inflammation/regeneration	Moura *et al*[36], 2019; Gondaliya *et al*[51], 2022
miRNA-18a/19a	Up	/	*TSP-1*	/	Proliferation	Wang *et al*[40], 2020
miRNA-15a-3p	Up	Mouse	*NOX5*	ROS	Proliferation	Xiong *et al*[41], 2020
miRNA-152-3p	Up	Mouse	*PTEN*	/	Proliferation	Xu *et al*[45], 2020
miRNA-133b	Up	Mouse	*EGFR*	/	Proliferation	Zhong *et al*[46], 2021
miRNA-195-5p	Up	Rat	*VEGFA*	/	Proliferation	Liu *et al*[42], 2021
miRNA-205-5p	Up	Rat	*VEGFA*	/	Proliferation	Liu *et al*[42], 2021
miRNA-199a-5p	Up	Rat	*VEGFA, ROCK1*	/	Proliferation	Wang *et al*[49], 2022
miRNA-203	Up	Rat	*IL-8*	AKT	Regeneration	Yuan *et al*[52], 2019
		/	*p63*	/	Regeneration	Liu *et al*[53], 2022
miR-489-3p	Up	Rat	*SIRT1*	PI3K/AKT/eNOS	Regeneration	Huang *et al*[47], 2022
miRNA-21-3p	Down	Mouse	*SPRY1*	FGF	Regeneration	Wu *et al*[56], 2020
miRNA-146a	Down	/	*AKAP12*	Wnt/β-catenin	Regeneration	Peng *et al*[57], 2022
		/	*TLR4*	NF-κB	Inflammation	Zhang *et al*[58], 2022

HIF-1α: Hypoxia-inducible factor 1 α; VEGF: Vascular endothelial growth factor; SDF-1α: Stromal cell-derived factor-1α; IL: Interleukin; TNF: Tumor necrosis factor; FGF7: Fibroblast growth factor 7; TSP-1: Thrombospondin-1; NOX5: NADPH oxidase 5; ROS: Reactive oxygen species; PTEN: Phosphatase and tensin homolog; EGFR: Epidermal growth factor receptor; ROCK1: Rho-related kinase 1; SIRT1: Sirtuin 1; SPRY1: Sprouty homolog 1; AKAP12: A-kinase-anchoring protein 12; TLR4: Toll-like receptor 4; NF-κB: Nuclear factor-kappaB; PI3K: Phosphoinositide 3-kinase; eNOS: Endothelial nitric oxide synthase.

LNCRNAS

LncRNAs are located in highly conserved genomic regions with spatially and temporally tightly regulated expression and dysregulated expression profiles as important markers of altered disease or developmental status. The main mechanism and function of lncRNAs are to act as competing endogenous RNAs (ceRNAs) for miRNAs, which interact with mRNA target base pairs to control various signaling pathways[59]. Another mechanism is by interacting with RNA-binding proteins[60]. Increasing evidence shows that lncRNAs play an important role in diabetic complications. LncRNA 3632454L22RiK can promote corneal epithelial wound healing in diabetic mice by sponging miR-181a-5p[61]. The regulatory role of lncRNA MIAT in diabetic cardiomyopathy has also been demonstrated [62]. These findings indicate an increased awareness of lncRNAs in diabetic complications.

Inflammation

The mechanism of lncRNAs in the inflammatory phase lacks enough evidence. LncRNA growth arrest specific 5 (GAS5) has been identified as a tumor suppressor that inhibits cell proliferation and promotes apoptosis[63]. GAS5 expression was markedly elevated in DFU wounds[64]. GAS5 promotes the M1 phenotypic polarization of macrophages through the upregulation of signal transducer and activator of transcription 1 (STAT1), leading to prolonged inflammatory phase and delayed wound remodeling and closure[64]. STAT1 signaling is exactly the central pathway that controls M1-M2 polarization in macrophages. Reduced GAS5 levels in wounds appear to promote healing by facilitating the conversion of M1 macrophages to M2 macrophages. Thus, targeting lncRNA GAS5 may contribute to efficient therapeutic interventions for impaired wound healing in diabetes.

Proliferation

GAS5 regulates the inflammatory process of wound healing and plays a part in the proliferative phase. During the proliferative phase, GAS5 activates the HIF-1α/VEGF pathway by binding to TATA box-binding protein associated factor 15, stimulating endothelial cell proliferation and angiogenesis and leading to accelerated DFU wound healing[65]. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a relatively well-studied transcript among lncRNAs. The role of MALAT1 has been reported in a variety of diseases, including renal tumors, osteosarcoma, and gestational diabetes[66-68]. MALAT1 protects endothelial cells from oxidative stress injury by activating the nuclear factor erythroid-2-related factor 2 (Nrf2) pathway. MALAT1 is markedly reduced in DFU-infected tissues, leading to insufficient HIF-1α/VEGF activation and impeding angiogenesis[69]. The exogenous uptake of exosome lnc01435 by vascular endothelial cells alters the subcellular localization of transcription factor yin yang 1 (YY1) and synergistically upregulates histone deacetylase (HDAC) 8 expression with YY1. HDACs are important components of the NOTCH signaling pathway with negatively regulated expression levels and thus affect endothelial cell function and angiogenesis[70,71]. In summary, targeting GAS5, MALAT1, and lnc01435 may help develop new therapeutic strategies to treat DFUs.

Regeneration

LncRNA H19, located on chromosome 11, exhibits negative regulation of diabetic wound healing. LncRNA H19 acts as a sponge for miR-29b and competitively represses miR-29b expression; therefore, it upregulates fibrillin 1 (FBN1), activates the transforming growth factor-β/Smad signaling pathway, and promotes ECM accumulation[72]. Connective tissue growth factor (CTGF) is a matricellular protein from the Cyr61/CTGF/Nov protein family, which interacts with ECM protein to mediate external signal transduction into cells through many subtypes of integrin receptors[73]. During the proliferative phase of diabetic wound healing, lncRNA H19 recruits the transcription factor SRF to the CTGF promoter region, activating CTGF and its downstream MAPK signaling pathway to accelerate fibroblast proliferation and wound healing[74]. These findings elaborate lncRNA H19 as a regulator in the regenerative phase of wound healing. A novel lncRNA MRAK052872, named lnc-upregulated in diabetic skin (URIDS), is involved in the mechanism of DFU wound healing. Lnc-URIDS is highly expressed in diabetic skin and dermal fibroblasts treated with advanced glycosylation end products. Lnc-URIDS binds to procollagen-lysine and 2-oxoglutarate 5-dioxygenase 1 (plod1), decreases plod1 protein stability, and leads to dysregulated collagen deposition and delayed wound healing[27]. LncRNA cancer susceptibility candidate 2 (CASC2) was originally discovered in an endometrial cancer study and is located on human chromosome 10q26[75]. Furthermore, CASC2 overexpression inhibited fibroblast migration and proliferation, suppressed apoptosis, and facilitated wound healing, especially in DFU mice. By contrast, miR-155 overexpression inhibited the function of CASC2[75]. Another study showed that HIF-1α inhibition reversed the effects of miR-155 downregulation on fibroblasts[76]. Evidently, lncRNAs have a considerable regulatory role in cellular functions during re-epithelialization and remodeling.

The mechanisms by which lncRNAs cause DFU and delayed wound healing are atypical inflammatory responses, impaired angiogenesis, impaired and abnormal ECM accumulation, and epithelial processes that regulate wound healing. The lncRNAs in DFU and delayed wound healing are listed in Table 2. These findings provide new information for the clinical treatment of diabetic chronic non-healing wounds.

CIRCRNAS

CircRNAs are a unique type of ncRNA derived from exons, introns, or intergenic regions that are covalently linked to produce a closed loop structure in the absence of 50 caps and 30 tails. CircRNAs are conserved among species owing to their resistance properties to RNase R. CircRNAs are involved in a wide range of biological processes, such as transcription and mRNA splicing, RNA decay, and RNA translation; the dysregulation of circRNAs leads to abnormal cellular functions and human diseases[77,78]. CircRNAs can also act as a miRNA sponge to inhibit miRNA function, which plays a crucial role in the pathogenesis of diabetes and its vascular complications[79]. Circ-PNPT1 and has_circ_0046060 promote the development of gestational DM by regulating trophoblast cell function or causing insulin resistance[80,81]. Circ-ITCH improved renal inflammation and fibrosis in diabetic mice by regulating the miR-33a-5p/SIRT6 axis[82]. CircRNAs are closely related to the development of diabetes and its complications. Studies on the role and mechanism of circRNAs in DFU and delayed wound healing are relatively few. Existing studies evaluated the regulatory role of circRNAs on angiogenesis and re-epithelialization.

CircRNAs protein kinase, DNA-activated, catalytic subunit (circ_PRKDC, has-circ-0084443) is involved in the promotion of keratinocyte proliferation and the suppression of keratinocyte migration during wound healing[83]. Circ_PRKDC negatively regulates keratinocyte migration *via* the EGFR pathway, impeding re-epithelialization and angiogenesis[84]. However, circ_PRKDC knockdown promotes epidermal keratinocyte migration *via* the miR-31/FBN1 axis[83]. This finding shows that

Table 2 Long non-coding RNAs in diabetic foot and wound healing

Name	Expression	Sponge	Animal	Target gene	Pathway	Phase	Ref.
GAS5	Up	/	Mouse	*STAT1*	/	Inflammation	Hu *et al*[64], 2020
		/	Mouse	*TAF15*	HIF-1α/VEGF	Proliferation	Peng *et al*[65], 2021
MALAT1	Down	/	/	*HIF-1α/Nrf2*		Proliferation	Jayasuriya *et al*[69], 2020
Lnc01435	Up	/	Mouse	*YY1, HDACs*	Notch	Proliferation	Fu *et al*[70], 2022
H19	Up	miRNA-29b	Mouse	*FBN1*	TGF-β/Smad	Regeneration	Li *et al*[72], 2021
	Up	/	Rat	*CTGF, SRF*	MAPK	Regeneration	Li *et al*[74], 2020
URIDS	Up	/	Rat	*Plod1*	VEGF/TGF-β	Regeneration	Hu *et al*[27], 2020
CASC2	Down	miR-155	Mouse	*HIF-1α*	/	Regeneration	He *et al*[76], 2022

GAS5: Growth arrest specific 5; STAT1: Signal transducer and activator of transcription 1; TAF15: TATA box-binding protein associated factor 15; MALAT1: Metastasis-associated lung adenocarcinoma transcript 1; YY1: Yin yang 1; HDAC8: Histone deacetylase 8; FBN1: Fibrillin 1; CTGF: Connective tissue growth factor; SRF: Serum response factor; URIDS: Upregulated in diabetic skin; Plod1: Procollagen-lysine and 2-oxoglutarate 5-dioxygenase 1; CASC2: Cancer susceptibility candidate 2; HIF-1α: Hypoxia-inducible factor 1 α; VEGF: Vascular endothelial growth factor; TGF: Transforming growth factor; Nrf2: Nuclear factor erythroid 2-related factor 2.

circ_PRKDC has therapeutic potential for skin wound healing. Shang *et al*[85] evaluated the effect of circ-Klhl8 in epithelial progenitor cells (EPCs) on diabetic wound closure by establishing an *in vivo* mouse model of total skin defect and found that circ-Klhl8 overexpression increases the therapeutic effect of EPCs to promote diabetic wound healing by targeting the miR-212-3p/SIRT5 axis. Altered circRNA expression can affect disease progression and wound healing in DFU (Table 3). Studies on circRNAs in various stages of DFU and wound healing are few and prompted the need for further research on functional circRNAs in the future to identify limitations in DFU treatment.

NCRNAS IN CELL THERAPY

The standard treatment for DFUs includes optimizing blood flow, debridement, infection control, and offloading. In standard treatment, only 50% of patients heal within 20 wk and 50% relapse within 18 mo; thus, efficient treatment for DFUs are urgently needed[86]. Cell-based DFU therapy is a new treatment intervention therapy studied in the last few years. Stem cells can affect ulcer healing through various pathophysiological processes, such as stimulating tissue repair, increasing ECM synthesis, and promoting angiogenesis in ischemic tissues[87]. Soluble factors and extracellular vesicles secreted by stem cells are active factors in diabetic wound healing. Extracellular vesicles from mesenchymal stem cells (MSCs) are considered an alternative treatment for immune disorders, including diabetes. Emerging evidence suggests that MSC-derived exosomes applied to the wound surface can promote angiogenesis and tissue repair[88]. MSC regenerative therapy is a novel tissue regeneration modality that accelerates wound healing in DFU and identifies patients at high risk of amputation[89]. Adipose-derived stem cells (ADSCs) have become an alternative to cell therapy owing to their abundance, subcutaneous location, easy accessibility, and longer culture time than bone marrow mesenchymal cells (BMSCs) and thus exert greater proliferation and differentiation capacity. Previous studies found that ADSC transplantation can promote foot wound healing in diabetic rats whereas stem cell transplantation may have clinical application in DFU treatment[90]. EPCs are the precursor cells of vascular endothelial cells that can be directed to the site of ischemic injury and form new vessels through proliferation and differentiation to promote wound healing[91]. Cell-derived exosomes loaded with ncRNAs have a therapeutic effect on refractory DFUs.

Gondaliya *et al*[51] revealed the therapeutic potential of miR-155 inhibitor-loaded MSC-derived exosomes in diabetic wound healing and demonstrated that wrapping miRNA and antibiotics in MSC-derived exosomes improved the management of chronic, non-healing diabetic wounds. Studies found that miR-129 may promote diabetic wound healing by balancing ECM synthesis and degradation through the inhibition of Sp1-mediated matrix metalloproteinase 9 expression[26]. A recent study also showed that miR-129 loaded in MSC-derived extracellular vesicles promoted wound healing *via* the downregulation of tumor necrosis factor receptor-associated factor 6[92]. Evidently, miR-129 is an important regulator of the proliferative and regenerative phases of wound healing and may be a biologically active molecule in MSC for DFU treatment. Xu *et al*[93] showed that miR-221-3p in EPC-derived exosomes accelerated skin wound healing in normal and diabetic mouse models. The latest study further demonstrated the mechanism of miR-221-3p in diabetic wound treatment[94]. MiR-221-3p

Table 3 Circular RNAs in diabetic foot and wound healing

Name	Expression	Sponge	Animal	Target gene	Phase	Ref.
Circ_PRKDC	Up	/	/	*EGFR*	Proliferation	Wang *et al*[84], 2020
	Up	miR-31	/	*FBN1*	Proliferation	Han *et al*[83], 2021
Circ_Klhl8	Down	miR-212-3p	Mouse	*SIRT5*	Proliferation	Shang *et al*[85], 2021

Circ_PRKDC: Circular RNA protein kinase, DNA-activated, catalytic subunit; SIRT5: Sirtuin 5; FBN1: Fibrillin 1; EGFR: Epidermal growth factor receptor.

overexpression may inhibit the anti-angiogenic function of its direct targeted homeodomain-interacting protein kinase 2 (HIPK2) and promote endothelial cell proliferation[94].

Li *et al*[95] showed that the MSC-derived exosomal lncRNA, lncRNA H19, causes fibroblast inflammation and apoptosis by disrupting miR-152-3p-mediated PTEN inhibition, leading to a stimulated wound-healing process in DFU. MSCs have demonstrated a therapeutic effect in DFU by generating pro-angiogenesis factors, such as VEGF. Recent research shows that genetically modified MSCs have been used in therapy, and the depletion of miR-205-5p in human MSCs promotes VEGF-mediated therapeutic effects in DFU[96,97]. LncRNA MALAT1 is a ceRNA for miR205-5p but has a low expression in human MSCs. Ectopic MALAT1 expression in human MSCs considerably decreased miR-205-5p levels, resulting in the upregulation of VEGF production and improved *in vitro* endothelial cell tube formation. In an immunodeficient NOD/SCID mouse model of diabetic foot (DF), the transplantation of human miR-205p-depleted MSCs resulted in better therapeutic effects on DF recovery than control MSCs. Moreover, MALAT1-expressing MSCs showed even better therapeutic effects on DF recovery than miR-205-5p-depleted MSCs. This difference in DF recovery was associated with the levels of on-site vascularization. Overall, MALAT1 functions as a sponge RNA for miR-205-5p to increase the therapeutic effects of MSCs on DF[97]. As mentioned above, miR-205-5p is an anti-angiogenic factor that inhibits VEGFA expression at the post-transcriptional level in MSCs, and the inhibition of its expression leads to angiogenesis and considerably improves the therapeutic effect of MSCs on diabetic wounds[97,98]. BMSC-derived exosomes can encapsulate lncRNA Kruppel-like factor 3 antisense RNA 1 (KLF3-AS1); adequately promote vascular endothelial cell proliferation, migration, and tube formation; and inhibit high glucose-induced apoptosis[99]. Diabetic wound healing by lncRNA KLF3-AS1 encapsulated by MSC-derived exosomes was achieved by downregulating miR-383 and upregulating its target, VEGFA[99].

High-throughput sequencing revealed an abnormally reduced expression of mmu_circ_0000250 in diabetic mice[100]. Exosomes from mmu_circ_0000250-modified ADSCs promote wound healing in diabetic mice through the induction of miR-128-3p/SIRT1-mediated autophagy[100]. In the study by Shi *et al*[100], the exosomes of ADSCs exerted therapeutic effects by restoring vascular endothelial cell function under high-glucose conditions. Circ-0000250 expression may increase the effectiveness of exosome therapy. Circ_ARHGAP12 is a cyclic molecule that inhibits high glucose-induced cell apoptosis by enhancing cellular autophagy[101]. Circ_ARHGAP12 was able to directly interact with miR-301b-3p and subsequently stimulate miRNAs to regulate the expression of ATG16L1 and ULK2, the target genes of miR-301b-3p, as well as downstream signaling pathways[101]. These findings propose a prospective therapeutic strategy of targeting circ_ARHGAP12 in MSCs to promote diabetic wound healing. Recent studies have found that circRNAs HIPK three (circHIPK3)-rich exosomes derived from human umbilical cord-derived MSCs have promising therapeutic effects in DFU. Exosomal circHIPK3 significantly promotes revascularization and wound healing by sponging to miR-20b-5p and upregulating the Nrf2/VEGFA axis[102]. Some ncRNAs for the cell therapy of DFU are shown in Table 4. NcRNAs and vector exosomes are effector molecules with great potential among the cellular therapeutic approaches for DFU and are expected to be of clinical use in the near future.

CONCLUSION

This study summarized the role and intrinsic mechanisms of ncRNAs in diabetic wound healing and provided more potential targets for future studies on wound healing in patients with diabetes. NcRNAs are regulatory molecules that modify many physiological processes and aspects of human diseases. The inflammation, proliferation, and regeneration phases of diabetic wound healing overlap, and ncRNAs are biologically active during all three phases. NcRNAs have a crucial role in the pathogenesis and impairment of wound healing in patients with diabetes. NcRNAs activate certain signaling pathways by downregulating or upregulating certain genes. Some of these molecules may provide valuable information in the clinical setting and serve as diagnostic or screening tools to predict high-risk DFUs and provide a basis for early prevention. These findings suggest that cell therapy using ncRNAs for DFU has great potential in the field of regenerative medicine.

Table 4 Non-coding RNAs in cell therapy

Name	Origin	Expression	Sponge	Target gene	Phase	Ref.
miRNA-155	MSC	Up	/	*FGF7*	Proliferation	Moura *et al*[36], 2019; Gondaliya *et al*[51], 2022
miR-129	MSC	Down	/	*TRAF6*	Proliferation	Hu *et al*[92], 2022
miRNA-221-3p	EPC	Down	/	*HIPK2*	Proliferation	Yu *et al*[94], 2022
LncRNA H19	MSC	Up	miRNA-152-3P	*PTEN*	Proliferation	Li *et al*[95], 2020
MALAT1	MSC	Down	miR-205-5p	*VEGF*	Proliferation	Zhu *et al*[97], 2019
Lnc KLF3-AS1	BMSC	Down	miR-383	*VEGFA*	Proliferation	Han *et al*[99], 2022
Circ_0000250	ADSC	Down	miR-128-3p	*SIRT1*	Proliferation	Shi *et al*[100], 2020
Circ_ARHGAP12	MSC	Down	miR-301b-3p	*ATG16L1, ULK2*	Proliferation	Meng *et al*[101], 2022
Circ HIPK3	MSC	Down	miR-20b-3p	*Nrf2/VEGFA*	Proliferation	Liang *et al*[102], 2022

MSC: Mesenchymal stem cells; FGF7: Fibroblast growth factor 7; TRAF6: Tumor necrosis factor receptor-associated factor 6; EPC: Epithelial progenitor cells; HIPK2: Homeodomain-interacting protein kinase 2; PTEN: Phosphatase and tensin homolog; SIRT1: Sirtuin 1; VEGF: Vascular endothelial growth factor; Lnc KLF3-AS1: LncRNA Kruppel-like factor 3 antisense RNA 1; BMSC: Bone marrow mesenchymal cells; ADSC: Adipose-derived stem cells; Circ HIPK3: Circular RNA homeodomain-interacting protein kinase three; Nrf2: Nuclear factor erythroid 2-related factor 2; MALAT1: Metastasis-associated lung adenocarcinoma transcript 1; Lnc: Long non-coding; miRNA: Micro RNA.

FOOTNOTES

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REVIEW

Diabetic foot ulcer: Challenges and future

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Abstract

Diabetic foot ulcers (DFUs) have become one of the important causes of mortality and morbidity in patients with diabetes, and they are also a common cause of hospitalization, which places a heavy burden on patients and society. The prevention and treatment of DFUs requires multidisciplinary management. By controlling various risk factors, such as blood glucose levels, blood pressure, lipid levels and smoking cessation, local management of DFUs should be strengthened, such as debridement, dressing, revascularization, stem cell decompression and oxygen therapy. If necessary, systemic anti-infection treatment should be administered. We reviewed the progress in the clinical practice of treating DFUs in recent years, such as revascularization, wound repair, offloading, stem cell transplantation, and anti-infection treatment. We also summarized and prospectively analyzed some new technologies and measurements used in the treatment of DFUs and noted the future challenges and directions for the development of DFU treatments.

Key Words: Diabetic foot ulcer; Diabetic peripheral artery disease; Diabetic peripheral neuropathy

Core Tip: Diabetes foot ulcer has become one of the important causes of mortality and morbidity of diabetes patients, and it is also a common cause of hospitalization, which brings a heavy burden to patients and society. The prevention and treatment of diabetes foot ulcer needs multidisciplinary management. We reviewed the progress in the clinical practice of diabetes foot ulcer in recent years, such as revascularization, wound repair, offloading, stem cell transplantation, anti-infection treatment. We also summarized and prospected some new technologies and measurements in the treatment of diabetes foot ulcer, and pointed out the future challenge and development direction of diabetes foot ulcer.

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INTRODUCTION

The prevalence of diabetes is gradually increasing: the global prevalence was estimated to be 9.3% (463 million people) in 2019 and is expected to increase to 10.2% (570 million people) in 2030 and 10.9% (700 million people) in 2045[1]. The prevalence rate of diabetes in Chinese people older than 18 years of age is 11.2%[2]. Diabetic foot ulcer (DFU) is one of the most serious and dreaded complications of diabetes. A total of 10%-15% of patients with diabetes may experience foot ulcers[3]. At least half of all amputations occur in patients with diabetes, and the most common cause is DFU infection. In a large cohort study of patients with DFU and patients with diabetes in China, the annual ulcer incidence rate among diabetic patients was 8.1%, the annual new ulcer incidence rate among patients with DFU was 31.6%, the amputation rate among patients with DFU was 5.1%, and the annual mortality rates among patients with diabetes and patients with DFU were 2.8% and 14.4%, respectively, during the 1-year follow-up period[4]. DFU is the main cause of hospitalization, amputation, deterioration of quality of life and disability of patients, which imposes a heavy economic burden on the medical and health system, and its economic burden ranks tenth among all diseases[5]. Therefore, the management of DFUs is particularly important. This article mainly introduces the risk factors and treatment of DFUs.

RISK FACTORS FOR DFU

The World Health Organization and the International Diabetes Federation define DFU as a serious complication of diabetes, mainly due to foot tissue ulcers and wounds caused by hyperglycemia, diabetic peripheral vascular disease and/or diabetic peripheral neuropathy[1]. DFU results from multiple factors. The risk factors for DFU must be addressed to reduce the rates of foot ulcers and amputation (Figure 1).

Neuropathy is a common complication of diabetes that occurs in 50% of patients with type 2 diabetes. Neuropathy is an important cause of ulcers. Long-term hyperglycemia leads to peripheral nerve fiber damage. Distal sensor motor peripheral neuropathy is the most common type. It manifests as distal, symmetric, and length-dependent multiple neuropathy[6]. Usually, small nerve fibers are damaged earlier than larger nerve fibers[7]. The dysfunction of small-fiber nerves leads to sensory changes, such as sensory dullness, acupuncture sensations, numbness, burning sensations, abnormal pain and other clinical symptoms. Sensory defects, such as defects in pain perception and temperature perception, are clinically called protective sensory deficits. The loss of protective sensation leads to a loss of sensitivity to injury and stimulation of the lower limbs, thus leading to continuous unconscious trauma, which tends to form ulcers. Usually, diabetic ulcers are found when blood stains are observed on the socks and floor, which portends an untimely diagnosis and treatment of the ulcers and aggravates the disease. Compared with patients with diabetes presenting without neuropathy, patients with diabetes presenting protective sensory loss have a 7-fold increased risk of developing DFUs[8]. The autonomic nerves will also be damaged. Dysfunction of the peripheral sympathetic nerves may lead to reduced sweating, dry skin, cracking and an increased risk of calluses complicated with peripheral arterial disease, and the appearance of symptoms increased. In the absence of peripheral artery disease, the dorsal foot vein expands, the foot feels warm, and some edema occurs. This situation places the patient's foot at high risk of ulceration. Biomechanical changes occur in the early stage of diabetic neuropathy[9]. Motor neuropathy causes an imbalance of foot muscle tissue, muscle weakness and atrophy and changes the normal foot dynamics and pressure distribution, leading to the loss of joint stability and the development of foot deformities such as claw toe, hammer toe, horseshoe foot, Charcot's ankle, arch changes, and plantar aponeurosis[10]. The increased shear stress and friction force increase the risk of foot ulcers, and when these factors are combined with the loss of protective sensation, the risk of foot

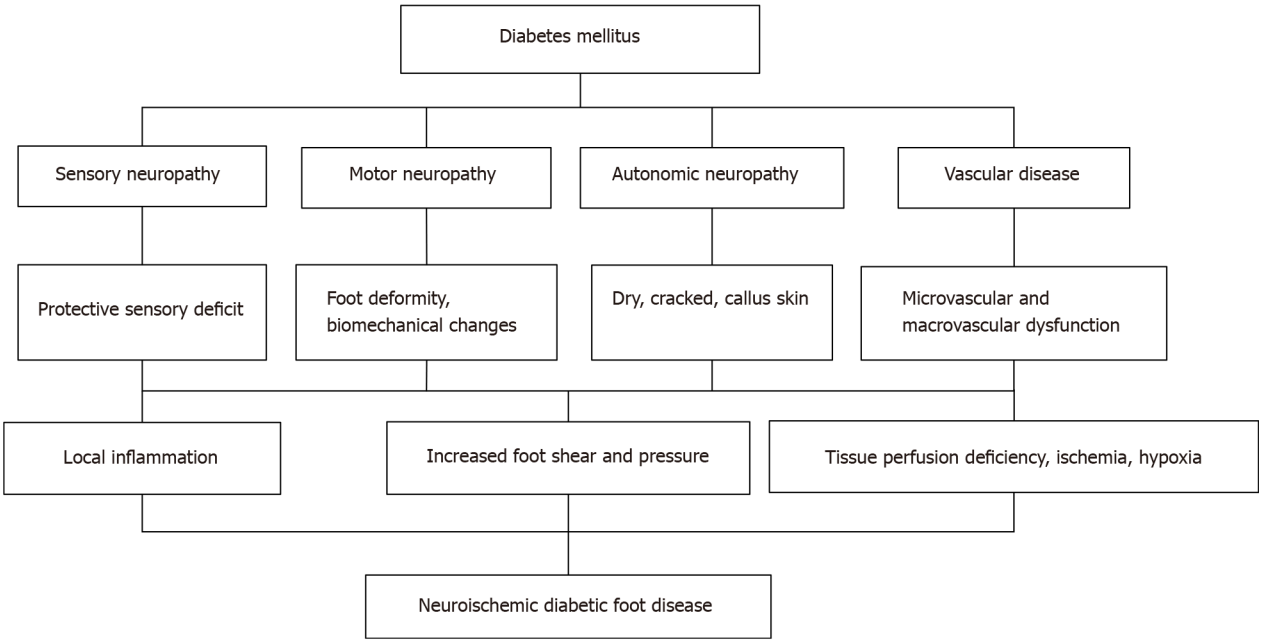

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Figure 1 Pathogenesis of diabetic foot ulcer.

ulcers is higher.

Lower extremity arterial disease is an important risk factor for DFUs, resulting in an insufficient blood supply, hypercoagulability of the lower extremities, and serious limb ischemia[11]. The clinical manifestations are malnutrition, muscle atrophy, decreased skin temperature, pigmentation, weakened or absent limb artery pulsation, and even intermittent claudication, resting pain and ulceration of the lower limbs. Long-term ischemia and hypoxia render the areas prone to tissue ulcers through the action of external forces, particularly ulcers at compressed parts of the heel or metatarsophalangeal joint, which are prone to secondary infection. Patients with diabetes usually have lower-limb arterial disease and neuropathy, which lead to difficult healing of neuropathic and ischemic ulcers. In 50%-75% of cases, peripheral arterial lesions lead to wound occurrence or a failure to heal[12,13].

The age and course of diabetes also affect ulcer healing. The risk of ulcer and amputation increases two to four times with increasing age and a prolonged disease course. Repeated minor injuries caused by neuropathic foot pressure and inappropriate footwear may increase the risk of ulceration. One study indicated that the overall risk of injury in patients with diabetes was 2% per year, the risk for patients with diabetes neuropathy increased to 7.5%, the risk for patients with a previous ulcer history increased to 40%, and the risk further increased to approximately 60% after 3 years and reached 75% after 5 years [14]. If the patient has eye diseases such as retinopathy and cataracts, resulting in decreased vision, the risk of a foreign body stabbing the foot is high, and the risk of ulceration is also increased. Some studies have suggested that dialysis patients with diabetic nephropathy have a very high risk of foot ulcers, and dialysis treatment is an independent risk factor for foot ulcers[15].

TREATMENT OF DFU

The ultimate goal of DFU treatment is to achieve healing and prevent wound infection, amputation and reduced quality of life. It mainly includes glycemic control, management of peripheral artery disease (PAD), and wound management, among others.

Glycemic control

Glycated hemoglobin may be the best indicator to evaluate average blood glucose control. An HbA1c level ≥ 8% and fasting blood glucose level ≥ 7 mmol/L are associated with an increased risk of lower limb amputation in patients with DFUs[16]. Studies have recommended that the glycated hemoglobin level in patients with DFUs should be controlled at 7%-8%, which is helpful for ulcer healing and will not increase the mortality of patients[17]. In another study, the glycosylated hemoglobin level was related to the wound healing speed, which was more obvious in patients with neuropathy and lower-limb arterial disease[18]; however, the results were not repeated in another study[19]. However, an appropriate blood glucose level is undoubtedly beneficial to prevent and delay microvascular and macrovascular complications in patients with diabetes[20]. The ideal blood glucose control target is

reached when the glycated hemoglobin level is less than 7% and the blood glucose level within 2 h after a meal is less than 11.1 mmol/L. However, the indicators should be appropriately relaxed for elderly patients and patients prone to hypoglycemia[21]. Regardless of the size of the initial ulcer area, early intensive blood glucose control may improve the prognosis of DFUs in the first 4 wk of treatment[22]. Intensive blood glucose control reduced the risk of amputation in patients with DFUs and contributed to wound healing[23,24]. However, in another systematic analysis, no evidence was obtained that strict control of blood glucose improved ulcer wound healing[25]. Many factors affect ulcer healing, and an increasing number of large samples randomized controlled trials (RCTs) are needed to indicate the effect of intensive blood glucose control on the prognosis of DFUs. According to the specific conditions of patients and blood glucose control objectives, appropriate hypoglycemic programs are formulated to avoid hypoglycemia. Fifty percent of patients with DFUs may have PAD, suggesting that they have atherosclerotic cardiovascular disease[26-28]. According to the latest recommendations of the American Diabetes Association for patients with type 2 diabetes complicated with cardiovascular disease, if blood sugar cannot be controlled by lifestyle changes and metformin, they should start taking a hypoglycemic drug that has been suggested to reduce adverse cardiovascular events and cardiovascular mortality[29], such as a sodium glucose cotransporter 2 inhibitor and a glucagon-like peptide-1 receptor agonist. Compared with the placebo, liraglutide did not increase the risk of DFUs but reduced the risk of DFU-related amputation in patients with type 2 diabetes mellitus and high-risk cardiovascular events[30]. However, the specific mechanism remains unclear. Studies have suggested that liraglutide may promote diabetic wound healing by inhibiting endothelial dysfunction induced by hyperglycemia[31]. Daggligin significantly reduced the level of inflammation and oxidative stress in diabetic animal models, which may contribute to the improvement of endothelial dysfunction and diabetic vascular complications[32]. However, some studies suggested that the amputation risk of patients who use canagliflozin is increased, particularly for patients with DFUs presenting lower limb atherosclerosis, neuropathy and amputation history[33]. However, the OBSERVE-4D study[34] indicated that although cagelin increases the risk of amputation, the risk islower than that reported in previous CANVAS and CANVAS-R trials [35], especially for patients undergoing timely monitoring, who have a lower risk. The results of a randomized controlled trial conducted by Marfella *et al*[36] suggested that the granulation score of wound granulation tissue and the rate of complete wound healing in the Vigliptin group (50 mg/dose, bid) were significantly better than those in the control group, and the incidence of ulcer-related adverse events (such as ulcer wound infection, osteomyelitis, honeycomb tissue inflammation, *etc*.) was also significantly reduced, suggesting that venagliptin may improve the healing rate of DFUs possibly by reducing oxidative stress, changing capillary density, increasing angiogenesis and promoting wound healing. Compared with the control group, the healing rate of foot ulcers in the saxagliptin (5 mg/time, qd) group was higher, and the healing time of ulcers was shorter. The main mechanism was that shagliptin directly and indirectly promoted the epithelial-mesenchymal transformation and reduced scarring to improve diabetic wound healing[37]. Dipeptidyl peptidase IV (DDP-IV) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists reduce inflammatory reactions and antioxidant activity, induce angiogenesis and tissue reconstruction, and may promote DFU healing[38]. These treatments represent new directions with potential effects on DFUs. Systemic insulin therapy improves wound healing of diabetic ulcers by increasing angiogenesis and granulation tissue formation and reducing the duration of the inflammatory phase[39]. Compared with patients with type 2 diabetes using insulin and insulin secretion-promoting drugs, patients with type 2 diabetes using insulin sensitizers have a lower incidence of PAD, suggesting that insulin sensitizers may reduce the incidence of PAD and its subsequent outcomes[40]. Therefore, when choosing hypoglycemic drugs, the appropriate hypoglycemic regimen should be selected according to the basic situation, blood glucose level, wound condition and other comprehensive factors of patients with DFUs.

Management of PAD

Patients with diabetes are prone to PAD. According to a Chinese multicenter study, the proportion of lower-limb arterial disease in patients with diabetes over 50 years old is 19.5%[41]. For every 1% increase in the glycated hemoglobin level, the risk of peripheral vascular disease increases by 25%-28% [42]. For patients with DFUs, the vascular lesions are mainly located in the tibiofibular artery below the knee. The arterial lumen is narrow or even completely occluded, causing lower limb ischemia, hypoxia, infection, ulcer and even gangrene. More than 80% of patients with DFUs have lower limb ischemia[43], which is an important reason for the difficulty in wound healing. Adequate blood perfusion provides a good metabolic demand for the damaged tissue, while an insufficient blood supply may lead to insufficient nutrients available for wound healing and limited delivery of antibiotics, resulting in a decreased healing capacity and increased amputation risk. Therefore, the arterial blood supply of the lower extremities must be reconstructed to improve and restore the blood flow of the extremities, avoid limb ischemia and necrosis, reduce amputation, and improve the quality of life and survival rate of the patients[26,27]. Patients with PAD have a high risk of cardiovascular and cerebrovascular events. Even after revascularization, the incidence of cardiovascular disease is still high[44]. Therefore, patients should also receive active cardiovascular risk management, including smoking cessation, statins, antiplatelet drugs and intensive blood pressure therapy[28,45]. Smoking is a risk factor for atherosclerotic plaques. Severe peripheral atherosclerosis may lead to stenosis and occlusion of the vascular

lumen with the progression of the disease, which may lead to foot tissue ischemia that causes tissue damage and postpones wound healing[46]. Smoking is also a risk factor affecting the degree of DFU lesions[47] and is an effective predictor of death and amputation in patients with DFUs[48]. Smoking cessation is recommended for all patients with PAD. Blood pressure should be controlled within 130/80 mmHg to reduce the risk of cardiovascular and cerebrovascular events[49]. However, another study suggested that the optimal mean systolic blood pressure of patients with PAD was 135–145 mmHg and diastolic blood pressure was 60–90 mmHg. Low blood pressure may increase the risk of cardiovascular events[50]. The use of angiotensin converting enzyme inhibitors (ACEIs) and angiotensin-receptor blockers (ARBs) by patients with PAD not only reduces blood pressure but also reduces major cardiovascular adverse events and mortality[51,52], but it does not reduce major adverse limb events and amputation risk in patients with PAD[52]. Treatments regulating lipid levels target low-density lipoprotein cholesterol. Researchers have recommended a low-density lipoprotein cholesterol (LDL-C) level < 1.4 mmol/L (< 55 mg/dL) or a decrease in the LDL-C level by 50%[53]. In patients with PAD, patients who took statins had an 18% reduction in the long-term risk of adverse prognosis of the lower limbs (such as symptom deterioration, peripheral vascular reconstruction and ischemic amputation) and a 17% reduction in the incidence of cardiovascular events compared with patients who did not take statins, indicating that statins not only reduce the risk of adverse cardiovascular events but also exert a positive effect on the limb prognosis of patients with PAD[54]. Therefore, patients with type 2 diabetes and PAD should be prescribed statins. Medications for improving the circulation of patients with PAD include vasodilator drugs, antiplatelet drugs and anticoagulant drugs. Vasodilator drugs include alprostadil injection, beraprost sodium, cilostazol, salgrel hydrochloride, buflomedil and pentoxifylline, which reduce blood viscosity and change hypercoagulability. Aspirin and clopidogrel are considered antiplatelet drugs. Current practice guidelines recommend the use of aspirin or clopidogrel alone as a method for the secondary prevention of cardiovascular events in patients with PAD[55,56]. Compared with aspirin alone, clopidogrel combined with aspirin significantly reduces all-cause mortality and cardiovascular events, but the risk of severe bleeding is increased[57]. The COMPASS study suggested that the absolute benefit of low-dose rivalsaban (2.5 mg bid) combined with aspirin (100 mg qd) in reducing the risk of cardiovascular events and all-cause mortality in patients with stable atherosclerosis seems to be greater than that of nondiabetic patients[58]. Compared with aspirin alone, low-dose rivalsaban combined with aspirin reduced major cardiovascular events and major limb adverse events. Rivalsaban alone only reduced major limb adverse events but did not significantly reduce major cardiovascular adverse events. However, the latter two schemes increased the risk of bleeding, mainly in the gastrointestinal tract, but the incidence of fatal bleeding or bleeding in key organs did not increase [59]. Routine use of proton pump inhibitors may reduce bleeding from gastroduodenal lesions[60]. Therefore, the combination of low-dose rivalsaban and aspirin provides a new therapeutic direction for patients with diabetes complicated with PAD, but further studies are necessary to determine which subgroups of patients may benefit.

Debridement and management of infection

Patients with diabetes have hypoimmunity, slow ulcer healing and a high infection rate. Infection often occurs when pus flows around the wound and the surrounding tissue is red and swollen. DFUs are usually chronic wounds. The bacteria on the wound surface produce biofilms that inhibit wound healing. Biofilms induce inflammation in the surrounding tissues and adversely affect the removal of bacteria by antibiotics or the host immune system[61]. Therefore, the wound must be thoroughly cleaned. Debridement is the first and foremost measurement of DFU treatment, which involves removing necrotic, inactivated or seriously polluted tissues from the wound surface to convert the wound into an acute wound and facilitate wound healing[62]. Debridement should be performed as soon as possible.

At present, many methods, such as surgical debridement, maggot debridement, high-pressure fluid irrigation and enzymatic treatment, have been developed, but surgical debridement is usually preferred. Amputation is necessary when the ulcer infection worsens or osteomyelitis occurs. For patients with lower limb ischemia, the time of debridement and revascularization must be evaluated. For patients with dry gangrene, the blood supply should be reconstructed first, and then wound debridement should be performed to promote wound healing. However, if wet gangrene or abscess formation is observed in the wound, debridement is preferred. More than half of patients with DFUs had wound infections at the time of the visit. Infection is an important reason for hospitalization of patients with diabetes and an important factor contributing to the nontraumatic amputation of the lower limbs[63].

In the initial stage of superficial DFU infection, gram-positive cocci are mainly detected, among which *Staphylococcus aureus* and *Streptococcus* are the most common organisms[64,65]. If chronic infection, extensive necrosis, wet gangrene, deep infection, long-term repeated use of antibiotics and other conditions exist on the wound surface, a mixed bacterial infection is usually present. Currently, the proportion of gram-negative bacterial infections is increasing, and the proportion of fungal infections is also increasing[66]. Due to the autoimmune status of the body, sanitary conditions, repeated hospitalization, frequent use and abuse of antibiotics, multiple microbial infections, insufficient arterial blood supply of the lower limbs and other reasons, the number of multidrug-resistant bacteria is increasing. The most resistant pathogen is methicillin-resistant *Staphylococcus aureus*[67]. Therefore, accurate identi-

fication of the pathogen causing the bacterial infection is essential for anti-infection treatment of DFUs. Once the infection is confirmed, pathogenic bacteria samples should be collected after the necrotic tissues are removed from the infected wound and before the use of antibacterial drugs. Pathogenic bacterial culture samples shall be obtained from deep tissues as far as possible and sent for culture immediately after the samples are collected. In addition, samples should be collected repeatedly during anti-infection treatment to identify pathogenic bacteria and guide the selection of antibiotics. Tissue biopsy is considered the most useful and standard technology, but it may cause the spread of infection and the loss of adjacent tissue structure of limbs; thus, it is not completely feasible. The collection of swab culture samples is easier, and any type of ulcer can be used. However, the cotton swab culture results usually include colonized bacteria, and the test results are not necessarily reliable[68]. Compared with the culture method, the molecular test method is more sensitive and reliable, with high accuracy and a fast test speed. It represents a powerful method or the identification of microbial colonies infecting chronic wounds and has a bright future in the convenient nursing and treatment of DFUs[69]. Molecular microbiological diagnostic techniques improve the prognosis of patients with chronic wounds[70].

The use of antibiotics should follow the principles of selectivity, timeliness, relatively narrow spectrum, shortest course of treatment, safety, minimal adverse reactions, high cost performance, and step-down. At present, the Infectious Diseases Society of America/International Working Group on the Diabetic Foot (IWGDF) is used to score DFU infection, which is divided into mild (superficial with slight cellulitis), moderate (deeper or more extensive) or severe (with systemic sepsis signs), and the presence of osteomyelitis[71].

The course of antibiotic use is related to the severity of the wound and the presence of bone tissue involvement. The course of treatment ranges from 1 to 12 wk[61]. However, a comprehensive and individualized analysis is necessary to appropriately adjust the course of antibiotics according to the basic diseases of the whole body, nutritional status, liver and kidney functions, blood supply of the lower limbs, and other parameters. Oral antibiotics and intravenous antibiotics maybe selected, but the narrowest-spectrum antibiotic and the shortest course of treatment for pathogenic bacteria should be selected to prevent drug resistance. The IWGDF recommendations[72] for superficial ulcers with localized soft tissue infection (mild) are to start with empirical oral antibiotic treatment against *Staphylococcus aureus* and *Streptococcus aureus* (unless other pathogens should be considered). For deep or extensive infections (moderate or severe infections), a broad-spectrum antibiotic should initially be intravenously administered that mainly targets common gram-positive and gram-negative bacteria, including specific anaerobic bacteria, and the antibiotic program should be adjusted according to the clinical efficacy of empirical therapy, tissue culture and drug sensitivity results. Biofilms are polysaccharide layers formed by a variety of signal transduction mechanisms that delay the healing of DFUs. Therefore, inhibiting the formation of biofilms is a new direction of modern anti-infection treatment research. Studies have shown that acyl homoserine lactones (AHLs) regulate multiple factors during biofilm formation by *Pseudomonas aeruginosa* and play a fundamental role in regulating different genes involved in biofilm formation. Therefore, AHL can be used as a therapeutic target to provide a correct path for drug design targeting multidrug-resistant bacteria[73]. However, in practice, the abuse of antibiotics still frequently leads to the emergence of drug-resistant bacteria. Biological maggot debridement therapy (MDT) provides a new option for the treatment of DFUs.

MDT refers to a natural biological therapy that uses medical maggots to help clean ulcerated wounds by eating the necrotic tissue and bacteria that hinder wound healing[74]; it is used for wounds with unclear boundaries between necrotic tissue and normal tissue, gaps or deep sinuses. It has anti-infection functions, promoting wound growth and debridement. However, because maggots need a moist living environment, ischemic DFUs are not suitable. Currently, the most commonly used organism is the larva of the green silk fly, which is strictly saprophytic and will not cause damage to healthy tissues[75]. Maggots resist infection by draining bacteria from the wound[76,77], absorbing and digesting bacteria in the necrotic tissue of the wound[78], changing the pH of the wound, secreting a variety of bactericidal and antibacterial substances (such as allantoin, urea, phenylacetic acid, calcium bicarbonate, peptides, and bactericides)[79-81], hindering the formation of and degrading bacterial biofilms[82], improving tissue oxygenation at the wound[83], and stimulating the growth of human fibroblasts[84]. Maggots also activate inflammatory cells and other mechanisms to promote wound tissue growth. The necrotic tissue can be cleared through the proteolytic enzymes produced by maggots (such as chymotrypsin, trypsin and collagenase)[85,86] to achieve the debridement effect. Studies have suggested that MDT is effective against a variety of bacterial infections, especially *Staphylococcus aureus*[87], which is resistant to multiple antibiotics. MDT reduces the number of bacteria and decreases the antibiotic treatment time and the hospitalization cost.

In the local treatment of wounds, antiseptics have more extensive antibacterial activity than antibiotics, and they do not induce drug resistance[88]. Antiseptics are commonly used to reduce the bacterial load of wounds and prevent or treat infections[89]. However, the finding that the antiseptic may be toxic to wound-healing cells is a cause for concern, which limits its application. Therefore, the current guidelines recommend the use of clean water or saline to clean the DFUs as the standard of care. In vitro studies of povidone iodine have shown that it penetrates and reduces the formation of biofilms, and it seems to have no negative effect on wound healing[90].

Dressing

After effective debridement of the wound, dressings are applied to keep the base of the wound moist, control the exudation of the wound, avoid normal skin impregnation around the wound, help clean the chronic wound, promote the formation of epithelium and heal the wound. The choice of wound dressings for patients with DFUs should be based on the patient's specific conditions (such as the wound appearance, depth, exudate, infection, compliance and economic status) and the cost and comfort of dressings. Hydrogel dressings, film dressings, foam dressings, hydrocolloid dressings, and alginate dressings are commonly used in clinical practice[91]; see Table 1 for detailed descriptions of various dressings. Hydrogel dressings are widely used in patients with DFUs. These dressings can expand and absorb water and exudate, maintain structural stability, and promote cell proliferation and differentiation and wound healing[92,93]. Some studies have shown that hydrogel dressings shorten the wound healing time by 7.28 d on average and improve the healing rate by 57%[94]. Alginate dressings absorb large amounts of water and can absorb 20 times their own weight. They are the best choice for highly exudative wounds[95]. Synthetic foam dressings can be selected for severe exudative wounds and concave wounds to fill cavities and eliminate potential cavities[96]. In an international, multicenter, double-blind, randomized, controlled, 20-week trial, the noninfectious diabetic neuroischemic foot ulcer had an area greater than 1 cm^2 compared with the control group receiving the same standard of care. After the use of sucrose octasulfate dressing, the number of patients exhibiting wound closure was greater, the wound healing time was shorter, the wound closure rate was improved, and the safety was similar between the two groups[96]. In 2019, the international national guidelines for the prevention and treatment of diabetic foot recommended the use of asucrose octasulfate dressing to promote wound healing of noninfectious neuro-ischemic DFUs that are difficult to heal after standard care[72]. Different dressings have their own advantages. They are selected in the clinic according to the specific conditions of the patient's wound and the characteristics of various dressings (Table 1).

Growth factors and platelet-rich plasma

Growth factors play an important role in wound healing. Vascular growth factors promote the formation of vascular collateral circulation and improve the blood supply of the lower limbs; these factors include fibroblast growth factor and hepatocyte growth factor[97,98]. Platelet-derived growth factor, which is mainly released from platelets, is also released from other cells involved in wound healing, such as endothelial cells, macrophages, keratinocytes and fibroblasts. Platelet-derived growth factor stimulates the secretion of vascular endothelial growth factor and promotes angiogenesis, fibroblast activity, granulation tissue formation and endothelial cell migration. However, a risk of tumorigenesis has been noted, and its application is limited. A systematic analysis was performed to study the effects of 11 different growth factors on DFUs. The local application of growth factors may increase the possibility of a complete cure of foot ulcers in patients with diabetes, but the quality of evidence is low[99]. The safety of using growth factors and the overall reports of their adverse events are very poor, and a comparison of the time points at which various growth factors promote wound healing has not been performed. Further tests are needed to study the effects of growth factors on wound healing. Platelet-rich plasma (PRP) is an autogenous product containing a large amount of platelets, growth factors, fibrin and other substances necessary for wound healing[100]. It is often used to treat relatively sterile wounds after debridement, which potentially improves the proliferation of local granulation tissue in ischemic wounds and promotes wound healing. Compared with standard treatment, topical application of PRP increases the healing potential and promotes complete wound healing without significant adverse events, although the quality of evidence is low[100]. Although growth factors and PRP may promote the healing of DFUs from the perspective of the path-ophysiological mechanism, greater requirements are present on the wound surface. Usually, DFUs are complicated with infections and bacterial biofilms exist, which limits the therapeutic application of growth factors and PRP.

Oxygen therapy

DFUs are often chronic wounds; as a continuous oxygen supply is essential for chronic and difficult wounds[101], oxygen therapy has emerged as a potential treatment. Currently, hyperbaric oxygen and local oxygen therapy are commonly used oxygen therapies. Hyperbaric oxygen therapy is applied *via* a hyperbaric oxygen chamber to reduce inflammatory reactions[102] and induce angiogenesis to promote wound healing, thereby reducing the amputation rate[103]. However, the efficacy of hyperbaric oxygen on DFUs is still controversial. In 1987, *Diabetes Care* published the first cohort study showing that hyperbaric oxygen significantly reduces the amputation rate of patients with DFUs[104]. Follow-up studies also found that when the traditional treatment method for chronic DFUs is not effective, hyperbaric oxygen treatment improves long-term wound healing[105,106]. However, in the study by Margolis, patients with DFUs who were treated with hyperbaric oxygen did not show significantly improved wound healing and amputation rates compared with the control group[107]. Dr. Fedorko *et al* [108] also confirmed that hyperbaric oxygen did not significantly improve the quality of life of patients with DFUs. At the same time, the results of two other RCTs evaluating the use of hyperbaric oxygen in the treatment of DFUs also showed that hyperbaric oxygen therapy did not reduce amputation or

Table 1 Introduction to common clinical dressings[91]

Type of dressing	Character	Scope of application	Advantage	Shortcoming
Film dressing	The polyurethane film is used as a protective layer or a second layer of dressing	Clean and superficial wounds	Good air permeability, isolating bacteria and liquid, transparent film, easy to observe the wound, less immersion, no pain	Strong adhesiveness, non-absorption, easy accumulation of wound exudates, leading to easy growth of bacteria and infections, and impermeability of proteins and drugs
Foam dressing	It is composed of polyurethane or a silicone resin center with a semi-closed outer layer	Burns, chronic wounds, cavity wounds, deep ulcers	Strong water absorption, local humid environment, free from bacteria, easy to use and low cost	Strong adhesiveness, forming an opaque layer, hindering wound observation, unsuitable for dry wounds, and poor stability
Hydrogel dressing	The composition is 70%-90% water and cross-linked insoluble starch polymer; super absorbent resin	Most wound and burn types	Supplement water to maintain a humid environment, high exudation, poor adhesion, easy to remove, accelerate wound healing, reduces pain and inflammation, and low cost	Translucent, semipermeable to gas and water vapor, poor bacterial barrier, sometimes poor mechanical stability, frequent replacement needed, and may cause secondary damage to the wound
Alginate dressing	Alginate is composed of calcium alginate and a calcium–sodium complex, forming a gel on the wound surface to promote Hemostasis	Surgical wounds and burns	Strong water absorption, non-adhesion, high stability, easy to be removed by salt water, and good bacterial barrier	Expensive, smelly, scarce materials, difficult to handle
Hydrocolloid dressing	It is composed of viscous materials, hydrophilic colloids, artificial elastomers, and other components that contact wound exudates to form gels and exert its functions	Chronic ulcers and burns	Strong water absorption, salt water or sterilized water is easy to clean and remove, not easy to adhere, high density, good waterproof performance, and no pain	Slight cytotoxicity, unstable volume, easy leakage of exudate, delayed healing of dextran hydrocolloid, impermeable, unpleasant smell, and obstructing wound observation

promote wound healing in patients with diabetes complicated with chronic DFUs through comprehensive wound care[109,110], but the results of these two studies were highly biased. At present, the number of studies assessing hyperbaric oxygen therapy for DFUs is small, the level of research evidence is low, the efficacy evaluation indicators are uneven, and many subjective factors are present. At the same time, hyperbaric oxygen is time-consuming, expensive, and cost-effective, which restricts the recommendation of hyperbaric oxygen therapy for DFUs. Local oxygen therapy directly delivers oxygen to the wound by pressurization. A multicenter randomized double-blind controlled study showed that standard treatment supplemented with local oxygen therapy increased the possibility of wound healing by more than 4.5 times[111], but more studies are needed to further confirm its efficacy.

Biological scaffold

Biological therapy has been used to promote diabetic wound healing. A study assessing the effect of biological scaffolds (chitosan polyvinyl alcohol and polycaprolactone chitosan polyvinyl alcohol nanofiber blend scaffolds) on the treatment of diabetic rats indicated that the scaffolds had higher biological performance. Compared with the control group, the ulcer area of diabetic rats treated with biological scaffolds was smaller at all time points, and the healing effect was significantly better. At the same time, more obvious granulation tissue was detected in the scaffold-treated wounds[112].

Stem cells/exosomes/cell matrix

As a new technology for the treatment of DFUs, stem cell transplantation may promote neovascularization of the ischemic limb and improve and restore the blood flow of the limb to achieve the goal of treating limb ischemia and the ultimate goal of promoting ulcer healing. Stem cells that have been used in preclinical and clinical research include umbilical cord blood mesenchymal stem cells, umbilical cord mesenchymal stem cells, placental mesenchymal stem cells, adipose mesenchymal stem cells and bone marrow mesenchymal stem cells, among which adipose mesenchymal stem cells are the most widely used cell type. In addition, genetically engineered SCs that overexpress certain cytokines exhibit different characteristics from conventional stem cells *in vivo*, providing a new direction for future clinical applications[113]. The most frequently studied stem cells are mainly found in bone marrow, adipose tissue, cartilage and bone tissue, umbilical cord blood and placenta, of which bone marrow is the most abundant source and these stem cells have a multidirectional differentiation potential, differentiating into osteoblasts, chondroblasts, adipoblasts, muscle cells and nerve cells. Bone marrow mesenchymal stem cells rebuild the local microcirculation[114], improve the blood flow of chronic ischemic limbs[115], provide media and sufficient nutrition for wound repair and remove local metabolites. Pilot research on local transplantation of bone marrow mesenchymal cells for the treatment of patients with vascular disease involving DFUs and lower limbs that failed vascular reconstruction showed that the transplantation of these cells significantly improved the percutaneous oxygen partial

pressure and toe brachial index of the patients, and the limb preservation rate was 81%[116]. Similarly, another study showed that bone marrow mesenchymal stem cell therapy significantly improves the painless walking distance and wound healing of patients with diabetes presenting lower limb vascular occlusion[117]. After 6 mo of treatment with adipose mesenchymal stem cells, approximately 2/3 of the clinical symptoms of lower limb ischemia in patients with diabetes were relieved (including resting pain and walking distance), and angiography showed a significant increase in collateral circulation[118]. A study of nondiabetic patients with lower limb ischemia who could not undergo vascular reconstruction also showed that adipose mesenchymal stem cell transplantation improved their lower limb percutaneous oxygen partial pressure and promoted the healing of local ulcers, proving that this therapy is also applicable to nondiabetic patients with lower limb ischemia[119]. After an intravascular injection of umbilical cord blood mesenchymal stem cells into rats with diabetic skin ulcers, neovascularization in the ulcer area was substantially increased on the third day, new granulation tissue appeared on the seventh day, and stratified squamous epithelial tissue appeared on the fourteenth day. Based on observations on the seventh and fourteenth days, the skin ulcer area was significantly reduced. The mechanism was that the injected umbilical cord blood mesenchymal stem cells promoted the secretion of keratin 19 by epithelial keratinocytes, participating in the formation of extracellular matrix [120]. Bone marrow mesenchymal stem cells not only improve vascular disease in the lower limbs of patients with diabetes but also promote the healing of ulcers[117,121,122]. Studies have shown that adipose mesenchymal stem cells also contribute to the healing of skin ulcers in diabetic mice[123,124]. At present, the mechanism of action of stem cells remains unclear. Generally, mesenchymal stem cells directly from new blood vessels through endocrine secretion from vascular endothelial cells and smooth muscle cells that participate in angiogenesis through paracrine vascular endothelial growth factor, basic fibroblast growth factor, hepatocyte growth factor, angiopoietin-2, angiopoietin-1 and other angiogenic factors[125]; improve the local microcirculation; increase the blood supply of the distal foot; and promote the healing of DFUs, but the mechanism still requires further study.

With the research and disclosure of the mechanism of action of stem cells, the development of stem cells or other cell derivatives with clearer mechanisms of action for DFU treatment has become a research hotspot. The application of these derivatives in DFU treatment shows efficacy and characteristics similar to those of stem cells. Among these derivatives, exosomes are the most popular. Exosomes are spherical or cup-shaped vesicles surrounded by double-layer membranes that are secreted by various cells. They exist in saliva, blood, milk, semen, blood and other tissue fluids. Exosomes carry a variety of signaling molecules and bioactive substances and participate in the occurrence and development of systemic immunity, intercellular communication, cell proliferation, cell migration, cell differentiation and metabolic diseases[126]. Previous studies have confirmed that mesenchymal stem cells exert therapeutic effects on DFU. They release exosomes through paracrine signaling, and exosomes, important mediators of intercellular communication, participate in the appellate cell process, which is one of the mechanisms by which mesenchymal stem cells exert their therapeutic functions. Exosomes secreted by mesenchymal stem cells are membrane vesicles with a diameter of 30-150 nm and a density of 1.10-1.18 g/mL[127]; they carry nucleic acid molecules such as miRNAs, cytosolic proteins and mRNAs and bind to receptors to mediate intracellular signal transduction and change cell functions. Exosomes secreted by adipose mesenchymal stem cells promote the proliferation of vascular endothelial stem cells, angiogenesis, wound granulation tissue formation, and growth factor expression and reduce the levels of inflammation- and oxidative stress-related proteins in a high-glucose environment[128]. Exosomes secreted by umbilical cord blood mesenchymal stem cells induce wound angiogenesis[129]. In a study that treated chronic wound skin of diabetic rats with exosomes secreted by PRP, exosomes effectively induced the proliferation and migration of fibroblasts and endothelial cells and improved the angiogenesis and re-epithelialization of chronic wounds[130]. Mesenchymal stem cell exosomes derived from menstrual blood also promote neovascularization and increase the amount of neovascularization in the skin of diabetic mice[131]. Exosomes are characterized by a simple structure, lack of replicability, lack of genetic material in the stem cell nucleus, shorter action time, smaller particle size for easy diffusion in vivo, and other properties. At the same time, *in vivo* studies have confirmed that they have similar biological activity to stem cells intreating DFUs. Therefore, stem cell derivatives with higher safety and simpler mechanisms of action will have better development prospects. However, exosomes are also associated with various problems, such as a high preparation cost, hampering large-scale production. Methods to produce uniform and reliable exocrine therapeutic drugs is an important research topic for the clinical application of exosomes in the future.

Due to the inconvenience caused by the characteristics of stem cells in preparations for the external use of stem cells, an increasing amount of research is devoted to the development of excipients that provide support for stem cells, such as collagen scaffolds and cell gels, to prolong the maintenance of the efficacy of preparations for the external use of stem cells. The extracellular matrix (ECM) is a noncellular three-dimensional polymer network composed of collagen, elastin, proteoglycan/glycosaminoglycan, laminin, fibronectin and other glycoproteins[132]. It provides extracellular scaffolds for cells and interacts with cells. The ECM directly or indirectly affects the shape, metabolism, migration, proliferation and apoptosis of cells. Studies have confirmed that it is closely related to immunity, inflammation, angiogenesis, wound healing and malignant transformation[133]; therefore, maintaining ECM homeostasis is very important for DFU healing. Hyaluronic acid is an important component of the

skin ECM. It affects many processes, such as cell migration, proliferation, inflammatory reactions and angiogenesis, in the proliferation stage of wound healing and plays an important role in wound healing and tissue repair[134]. However, the hyaluronic acid content is reduced in DFU skin, resulting in delayed wound healing[135]. Collagen promotes myofibroblast differentiation and fibrosis to maintain the ECM structure and promote healing[136], but collagen deposition in DFU skin reduces the skin thickness and integrity[137]. In individuals with diabetes, the production of matrix metalloproteinases in the ECM increases, and the ratio of matrix metalloproteinases/tissue inhibitors of metalloproteinases increases, aggravating the inflammatory response and leading to an imbalance in ECM homeostasis [138]. In DFU wound tissues, the secretion of matrix metalloproteinases is increased, and the levels of matrix metalloprotease-hydrolyzed ECM fragments are increased. Studies have confirmed that the occurrence of inflammation *in vivo* is significantly related to the presence of a large number of ECM fragments and their receptors[139]. At the same time, the production of ECM fragments in the inflammatory process also activates immune cells, leading to the continuous occurrence of inflammatory reactions[140]. The interaction between the abnormally expressed ECM and the inflammatory response causes a high level of inflammation to persist in DFU wounds for a long time and makes wound healing difficult. In view of the complexity and safety of the stem cell therapy mechanism and the special disease characteristics of DFUs, the development of stem cell preparations for the external treatment of DFUs, such as those combined with ECMs caffolds, is another effective technical approach showing considerable application prospects and will certainly play an important role in future clinical applications.

Negative pressure wound therapy

Negative pressure wound therapy (NPWT) includes two modes: vacuum-assisted closure (VAC) and vacuum sealing drainage (VSD). The pipeline used by VAC has poor hydrophilicity and a high supporting force. The pipeline is placed on the surface of the dressing to form a device similar to a suction cup. Wound healing is promoted by adjusting the negative pressure level and selecting the intermittent mode[141]. The drainage tube adopted by VSD has high plasticity, good hydrophilicity and contains side holes. It covers the dressings and wound surface with a fully closed and translucent polyurethane film to form a closed space. The necrotic tissues and secretions on the wound surface are drained by negative pressure to promote wound cleaning; it is mainly used for drainage of deep wounds and body cavities. VAC focuses on the treatment of wounds on the body surface and exerts a good effect on treating DFUs, limb soft tissue lacerations, lower limb venous ulcers, deep pressure ulcers, and other wounds[142-144].

NPWT is widely used to treat DFUs as an acute and chronic wound treatment technology. Armstrong *et al*[142] suggested that, compared with standard treatment, VAC accelerated wound healing, improved wound healing ratio and reduced the re-amputation rate when treating complicated diabetic foot wounds. In the meta-analysis by Liu *et al*[145], compared with conventional dressing changes, VAC reduced the area and depth of DFU to a greater extent, improved the complete healing rate of ulcer, shortened the healing time of ulcer, reduced the amputation rate of patients, and improved cost-effectiveness.

The mechanism of NPWT is as follows: (1) Keep the wound moist and stabilize the wound environment pain[146]; and (2) inhibit bacterial growth. Weed *et al*[147] indicated that after treatment with negative pressure drainage technology, the number of bacteria in the wound, particularly gram-negative bacteria, was significantly reduced. Additional components of the mechanism include: (1) Improving wound blood perfusion and promoting wound healing[148]; (2) promoting cell proliferation, angiogenesis and wound tissue repair[149]; and (3) regulating the signaling pathway to modulate cytokine expression[150].

Before using NPWT, the necrotic tissue and dead bone on the wound surface should be completely removed. NPWT also has contraindications, such as deep wound infection, severe ischemia, eschar or necrosis, active bleeding, coagulation dysfunction, exposure of blood vessels or nerves or tendons or ligaments, untreated osteomyelitis, wet gangrene, and malignant tumors. At the same time, the use of NPWT may lead to tube plugging, poor drainage, residual dressings, wound maceration, residual dressings, and other complications.

Based on the limitations of NPWT, negative pressure wound therapy with installation (NEWTi) emerged at a historic moment. It combines negative pressure therapy with liquid perfusion technology to accelerate the cooperative use of wound water and promote the dissolution and clearance of deep necrotic tissues by intermittently or continuously perfusing solutions to closed wounds. The destruction of biofilms and autolytic debridement are the main factors contributing to the superiority of NPWTi to NPWT. However, a uniform standard for the selection of irrigation solution, irrigation time, irrigation speed, and irrigation frequency is unavailable when NPWTi is used to treat DFUs.

Offloading

At present, the basic principle for the prevention and treatment of neurogenic DFUs is to redistribute the increased local pressure on the foot[151,152], reduce the plantar pressure and shear force, and promote wound healing. Therefore, the selection of a suitable decompression device according to the actual situation of the patient is very important to prevent foot ulcers[153]. Offloading is divided into

nonsurgical offloading and surgical offloading. Nonsurgical offloading provides external decompression through the use of individually customized or prefabricated devices. The efficacy is determined by whether the patient can continue to wear the decompression device. Currently, the most commonly used pressure reducing devices are the total contact cast (TCC) and detachable cast. TCC is the most effective decompression technique for the treatment of neurogenic DFUs[154] and is even the gold standard for foot restraint and treatment of DFUs[14]. Studies have shown that TCC can reduce the pressure at the ulcer by 84%-92%[155], and it is effective for most nonischemic and noninfectious diabetic plantar ulcers, with an ulcer healing rate of 69.6%-73.9%[156,157]. A TCC can reduce local inflammation, help reduce or delay edema during wound healing, accelerate ulcer healing, and possibly protect the foot from infection because it is not easy to disassemble, which increases the patient's compliance with use and may be an important reason for its benefit[158,159]. However, as TCCs are not easy to disassemble and patients may easily fall while wearing them, their use limits patients' daily activities. Moreover, inappropriately fitting braces may cause skin irritation, even skin ulceration and infection, and muscle weakness may occur after long-term use. At the same time, TCC use requires the cooperation of experienced doctors, technicians and patients, and consequently, the application of TCCs is limited. The detachable plaster branch has the advantages of easy disassembly, easy observation of wounds, convenient local treatment of wounds, and it can be used for the treatment of infected wounds and superficial ulcers. However, it also has the disadvantages of poor patient compliance and the reduction of the local decompression effect due to irregular wearing. Therefore, patients must be educated while repeatedly emphasizing the benefits of consistently wearing the device to achieve the therapeutic effect[160]. For patients who do not accept gypsum braces, felt-like foam pads and appropriate therapeutic shoes can also be used for decompression treatment[72]. Studies have confirmed that therapeutic shoes reduce plantar pressure and prevent ulcer recurrence[161]. Crutches, walking aids and wheelchairs may also be used for decompression, but some devices increase the pressure on the healthy foot during use, which will increase the incidence of new ulcers on the healthy side[155]. At the same time, the use of these devices is limited due to the lack of upper limb strength and perseverance to use these devices independently[162].

Surgical decompression treatment serves to redistribute the pressure or change the position of the pressure points through surgery, with the purpose of permanently changing the internal pressure point. When a patient does not exhibit complete local decompression after using the optimized shoes and tools and ulcers occur or the patient cannot decompress after ulcer healing and after using the optimized shoes and tools, then he or she can be decompressed during amputation. Surgical decompression usually includes Achilles tendon extension, metatarsal head resection, arthroplasty, and toe flexor tendon resection[163]. Patients with diabetes are prone to shortening of the calf gastrocnemius muscle, which will lead to a continuous increase in the plantar pressure of the forefoot. The extension of the Achilles tendon may reduce the plantar pressure of the forefoot[164]. Studies have confirmed that Achilles tendon lengthening promotes wound healing and reduces ulcer recurrence in patients with neuropathic plantar ulcers and horseshoe foot[165]. Diabetic neuropathy leads to a high pressure load on the plantar skin above the metatarsal head. Removing these biomechanical factors may reduce pressure and facilitate wound healing. For nerve plantar ulcers with difficult healing, early removal of the metatarsal head may be the key to promoting wound ulcer healing[166]. Compared with traditional conservative treatment, metatarsal head resection has a higher healing rate and lower infection rate and ulcer recurrence rate[167]. At the same time, metatarsal head resection promotes the healing of plantar ulcers, which is not related to sex, age, body mass index, height, weight, diabetes duration or the duration of preoperative ulcers[168]. Arthroplasty is an effective procedure for the treatment of recurrent or complex neurological DFUs. Using routine treatment and decompression, metatarsal finger arthroplasty results in a faster healing rate and a lower recurrence rate than the standard treatment [169]. The flexor pollicis longus and flexor digitorum longus can be used to decompress the toe and make the toe tip more flexible[170]; a systematic review confirmed that this operation exerts a good therapeutic effect on closing wounds and newly formed ulcers[171].

Low-level laser therapy

Low-level laser therapy (LLLT) uses low-energy light to stimulate the wound surface and produce a series of pathophysiological reactions mainly through photobiological regulation. It does not directly induce photothermal injury of the wound tissue and does not damage the normal tissue cells at the wound surface[172]. In the study by Kaviani *et al*[173], 8 patients in the LLLT group achieved complete healing after 20 wk, while only 3 patients in the control group achieved complete healing. Although the difference was not statistically significant, the average time of complete healing in patients receiving LLLT (11 wk) was less than that in the control group (14 wk), suggesting that LLLT might accelerate the healing process of chronic DFUs and shorten the time of complete healing, but the sample size of this experiment was small. Another analysis of the efficacy of LLLT in the process of chronic wound tissue repair of DFUs showed that the tissue repair index in the LLLT treatment group increased significantly, mainly because LLLT shortened the inflammatory period, promoted angiogenesis and the production of extracellular matrix components, and accelerated the healing process[173,174]. Percival *et al*[175] proposed that LLLT promotes wound healing by inhibiting the microbial membrane of chronic wounds, especially cocci and some gram-negative bacteria. Other studies have shown that LLLT promotes

wound healing by improving the blood flow and autonomic nervous system regulation of DFUs[176]. A systematic review and meta-analysis of the efficacy of low-dose laser treatment of DFUs[177] found that the ulcer area in the LLLT treatment group was significantly reduced by 30.90% compared with the control group. Compared with the control group, the ulcer area in the treatment group decreased by 4.2 cm^2. The probability of complete healing of DFUs was 4.65 times higher than that of the control group, indicating that LLLT may accelerate wound healing and reduce the area of DFUs. However, the review did not provide the best laser treatment parameters. However, in another review, LLLT was shown to be safe and effective in treating DFUs. The laser parameters were 632.8-685 nm, 50 mW/cm^2, and 3–6 J/cm^2; the irradiation time was 30–80 s, three times a week, and the duration of one month was beneficial for the prognosis of DFU wounds in patients[178]. Because the pathophysiological mechanism of DFUs is complex and the prognosis of the ulcer surface is different due to the diverse ulcer surfaces and different laser parameters, more rigorous, high-quality and large-sample RCTs are needed to determine the best treatment parameters for different types of ulcers.

PREVENTION

The incidence of DFUs is high, and the amputation rate is high; moreover, ulcer healing is slow, and the treatment effect is relatively poor. Therefore, the prevention of DFUs is particularly important. However, people currently focus on treatment after the occurrence of DFUs. Researchers mainly focus on medical treatment rather than prevention. Cesare Miranda[179] suggested that comprehensive management should be implemented to prevent DFUs and provided a flow chart for the prevention of DFUs, including DFU education, blood glucose control, management of PAD, identification of risky feet, regular inspection of susceptible feet, long-term wearing of appropriate shoes, and treatment of ulcer risk factors. DFU education can reduce the incidence of DFUs and amputations. It encourages patients to conduct foot self-examinations, identify risk factors, provide appropriate self-care and treat feet with any signs of pre-ulceration[153]. However, the smooth performance of this examination is usually affected by the decrease in vision and limited movement of patients. Through regular foot screening and follow-up of patients with diabetes, the incidence of DFUs and the amputation rate have significantly decreased, but only 20%-30% of patients in China undergo regular foot screening[180]. Studies have shown that the use of diabetic foot treatment shoes and insoles may reduce the ulcer recurrence rate by 30%-50%, but the ulcer recurrence rate is still as high as 30%[181]. In the study by Frykberg *et al*[182], a new type of remote wireless intelligent temperature monitoring foot pad system was provided for patients with previous DFUs, which is a wireless temperature foot pad that can be used in daily life and senses changes in and asymmetry of foot temperature. The research results show that the intelligent detection system accurately predicts the experimental patients with recurrent DFUs. However, this experiment has its own limitations, including its nonintervention design, small sample size, short experimental time, lack of evaluation of other factors and costs that may affect the occurrence of DFUs, and artificial bias. The results thus require further confirmation. Nevertheless, the results of this study are still very meaningful, suggesting that more intelligent devices can be further developed for the prevention and treatment of DFUs and can thereby reduce the familial and social burdens related to patients with DFUs.

CONCLUSION

DFUs are one of the serious complications of diabetes. Many risk factors lead to the occurrence of the disease, and the amputation rate is high. Once diabetes is diagnosed, we should perform more work on the management of diabetes, including screening for high-risk factors for DFUs, such as neuropathy and arteriopathy. The high incidence of DFUs may be related to the lack of DFU risk education programs, the insufficient attention of patients, the low rate of foot examination, and the poor knowledge of medical personnel. Boulton *et al*[183] found that less than 20% of patients with diabetes received a foot examination provided by medical and health professionals. Therefore, foot care education should be provided to all patients with diabetes and the risk of DFU should be evaluated at a minimum of annually[184].

With the development of artificial intelligence, intelligent detection instruments and evaluation tools (such as intelligent insoles) can be applied to the prevention and treatment of DFUs. The management of DFUs requires multidisciplinary cooperation, mainly including endocrinologists, vascular surgeons, orthopedic doctors, wound specialists, shoe technicians, rehabilitation physicians, psychological consultants and specialized nurses. The correct evaluation and comprehensive management of DFUs by multidisciplinary teams are essential to protect the function and quality of life of patients. Optimizing diabetes management is still the most important step to prevent diabetes-related complications[185, 186]. The effect of intensive treatment on the prognosis of DFUs requires further study. In patients with diabetes complicated with PAD, sodium glucose co-transporter 2 inhibitors or GLP-1 receptor agonists are recommended[145,187]. Both DDP-IV inhibitors and GLP-1 receptor agonists promote DFU healing

[187]. When patients with DFUs choose hypoglycemic drugs, they should not only consider the hypoglycemic effect but also consider whether cardiovascular risk factors are present and whether these drugs can promote ulcer healing. Statins, antiplatelet agents, ACEIs and ARBs are effective in the secondary prevention of cardiovascular events in patients with PAD. For patients with PAD complicated with diabetes, the combination of low-dose rivarsaban and aspirin reduces major limb adverse events, including amputation[60], but the risk of bleeding must be monitored. Offloading relieves plantar pressure and shear force to promote wound healing. The value of decompression shoes lies in preventing ulcers, not in using them during the treatment of active ulcers[188]. Surgical offloading is mainly employed to treat specific foot ulcers, usually when other nonsurgical offloading interventions fail. Internal offloading and external offloading are used together to promote wound healing. Necrotic tissue and the microbial membrane are removed, and chronic wounds are transformed into acute wounds. A systematic review reviewed the effect of surgical debridement on DFU healing. The results indicated that the higher the application frequency of surgical debridement, the better the results[189]. However, excessive debridement is not conducive to ulcer healing. An appropriate debridement frequency and debridement method should be selected for different DFUs. The combined use of antibiotics, wound dressings and NPWT may accelerate wound healing. However, the efficacy of oxygen therapy must be confirmed in more high-quality studies. Additionally, the specific parameters of LLLT treatment for different DFUs also require strict, large-sample RCTs researches to provide data. Biological scaffolds, stem cells, exosomes, cell matrix, growth factors and PRP represent new approaches for the treatment of DFUs. The preliminary data seemed positive and revealed a potential effect, but the specific mechanisms of action of these therapies are not clear, and further clinical research may provide better suggestions. In summary, multidisciplinary combination treatment should be adopted in the treatment of DFUs.

The current situation is that the screening rate and follow-up rate of DFUs are low, the incidence rate and the amputation rate are high, and many treatment methods are available, but the effect is not satisfactory. However, with the development of the information age, people's understanding of diabetes and DFU has gradually improved, and various new technologies have been continuously developed, which provides opportunities for the management of DFUs. In the future, comprehensive prevention and treatment management of DFUs are needed to avoid the occurrence of DFUs, effectively shorten the healing time of DFUs, improve the clinical cure rate, reduce the amputation rate, improve the standard of living of patients with DFUs, and reduce the social burden. This task may be a complex, huge and meaningful project.

FOOTNOTES

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REVIEW

Keeping an eye on the diabetic foot: The connection between diabetic eye disease and wound healing in the lower extremity

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Abstract

Diabetic eye disease is strongly associated with the development of diabetic foot ulcers (DFUs). DFUs are a common and significant complication of diabetes mellitus (DM) that arise from a combination of micro- and macrovascular compromise. Hyperglycemia and associated metabolic dysfunction in DM lead to impaired wound healing, immune dysregulation, peripheral vascular disease, and diabetic neuropathy that predisposes the lower extremities to repetitive injury and progressive tissue damage that may ultimately necessitate amputation. Diabetic retinopathy (DR) is caused by cumulative damage to the retinal microvasculature from hyperglycemia and other diabetes-associated factors. The severity of DR is closely associated with the development of DFUs and the need for lower extremity revascularization procedures and/or amputation. Like the lower extremity, the eye may also suffer end-organ damage from macrovascular compromise in the form of cranial neuropathies that impair its motility, cause optic neuropathy, or result in partial or complete blindness. Additionally, poor perfusion of the eye can cause ischemic retinopathy leading to the development of proliferative diabetic retinopathy or neovascular glaucoma, both serious, vision-threatening conditions. Finally, diabetic corneal ulcers and DFUs share many aspects of impaired wound healing resulting from neurovascular, sensory, and immunologic compromise. Notably, alterations in serum biomarkers, such as hemoglobin A1c, ceruloplasmin, creatinine, low-density lipoprotein, and high-density lipoprotein, are associated with both DR and DFUs. Monitoring these parameters can aid in prognosticating long-term outcomes and shed light on shared pathogenic mechanisms that lead to end-organ damage. The frequent co-occurrence of diabetic eye and foot problems mandate that patients affected by either condition undergo reciprocal comprehensive eye and foot evaluations in addition to optimizing diabetes management.

Key Words: Foot ulcer; Diabetic; Wound healing; Diabetes complications; Amputation; Diabetic retinopathy; Corneal ulcer

Core Tip: This review explores the epidemiological and pathophysiological interconnections between diabetic foot and eye disease, especially the shared mechanisms that impact wound healing. Since diabetic foot and eye problems are often concurrent, it is imperative that patients affected by one or the other condition promptly undergo reciprocal examinations to reduce the risk of further complications. The best outcomes for patients with diabetic foot and eye disease are achieved by a team-based strategy that incorporates regular examinations, often performed by specialists, provides preventative health education, and delivers effective long-term management of the underlying diabetes and its associated metabolic consequences.

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INTRODUCTION

An estimated 131 million people worldwide have lower extremity complications related to diabetes mellitus (DM), such as diabetic foot ulcers (DFUs), peripheral vascular disease (PVD), neuropathy, and amputations[1,2]. Similarly, an estimated 103 million people worldwide have diabetic eye disease, including nearly one million people aged 50 and older who are blind from diabetic retinopathy (DR)[3,4]. The frequent co-occurrence of diabetic eye and foot problems makes it imperative for patients affected by either condition to promptly undergo reciprocal examinations to reduce the risk of further complications (Figure 1). It is essential that individuals who have DM and the clinicians who care for them understand the likelihood of this association. With the worldwide prevalence of DM increasing because of changes in diet and lifestyle, aging of the population, and the ability of individuals to live longer with the disease, the need for well-informed clinicians has never been greater[3].

The connection between diabetic eye and foot problems is related, in part, to shared risk factors. In particular, the duration of DM and level of glycemic control as reflected by hemoglobin A1c (HbA1c) level strongly govern both the rate of onset and severity of diabetic foot disease[5,6] and DR[7]. Molecular biomarkers, particularly ceruloplasmin, have been demonstrated to be elevated in people with DM[8]. Other risk factors, such as age, male gender, race and ethnicity, smoking, insulin use, type of diabetes, and individual comorbid factors such as hypertension, elevated low-density lipoprotein (LDL), decreased high-density lipoprotein (HDL), coronary artery disease, cerebral vascular disease, PVD, neuropathy, and nephropathy, have been assessed, but not all studies agree on which of these risk factors affect the incidence or progression of these diabetic complications[5]. Some of this variation may possibly be ascribed to differences in DM care, improvements in treatment over time, and other less well-defined differences between individual populations studied. This paper reviews the shared pathogenic mechanisms underlying these conditions and the importance of comprehensive diabetes care to reduce morbidity and prevent disability.

DIABETES-ASSOCIATED LOWER EXTREMITY COMPLICATIONS AND THEIR OCULAR PARALLELS

DFUs

Individuals with DM are at a significantly increased risk of developing DFUs. DFUs are full-thickness wounds that penetrate the dermis (the deep vascular and collagenous inner layer of the skin) and are located below the ankle in patients with both type 1 and type 2 DM (Figure 2)[1,2]. DFUs arise from a combination of micro- and macrovascular compromise related to hyperglycemia and associated metabolic dysfunction that causes impaired growth and wound healing, immune dysregulation, and PVD[9]. In addition, the loss of protective sensation and proprioception caused by diabetic neuropathy and vision loss from diabetic eye disease predisposes patients to repetitive lower extremity trauma, with DFUs a common complication, especially among older adults[10,11]. Risk factors for DFUs include age,

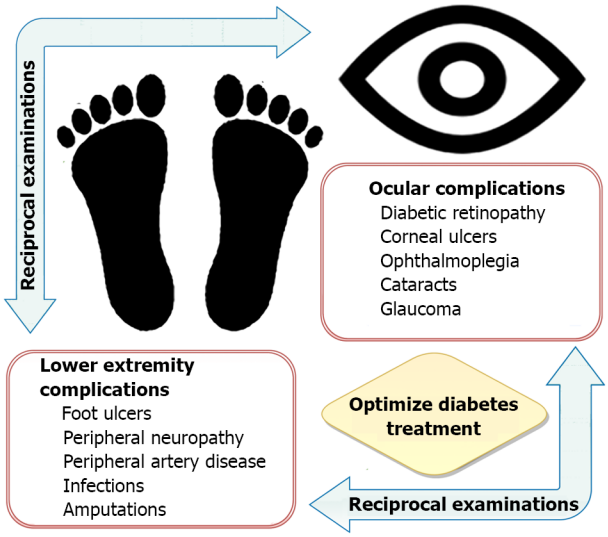

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Figure 1 Upon identification of one or more of problems involving the lower extremity or the eye, reciprocal examinations are recommended to reduce the risk of further complications. Preventing diabetic foot and eye problems is best achieved through regular examinations, diabetes education, and optimal management of underlying diabetes mellitus and its associated metabolic consequences.

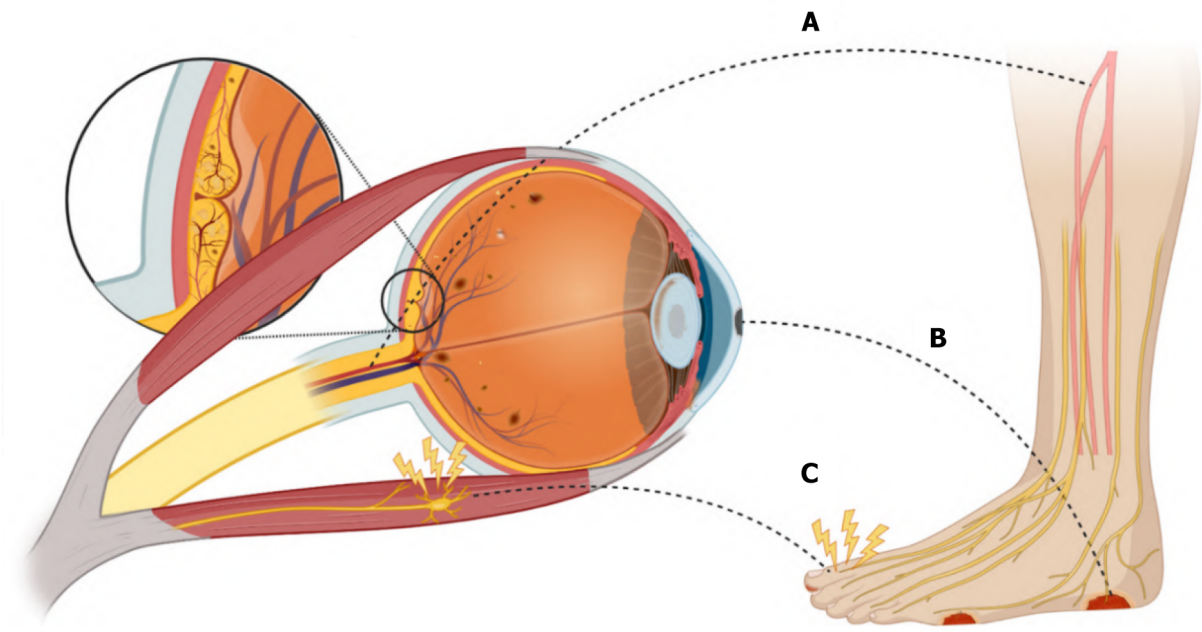

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Figure 2 The connection between diabetic eye disease and diabetic foot and wound healing. A: Diabetic micro- and macrovascular complications; B: Diabetic ulcers; C: Diabetic neuropathy. Each of these diabetic complications has multifactorial etiologies. Figure 2 was made in ©BioRender-biorender.com.

deformity or prior ulceration, repetitive trauma, sensory and autonomic neuropathy, peripheral arterial disease (PAD), and infection[12]. Up to one third of patients with DM will be affected by a DFU in their lifetime[13]. DFUs are associated with a 10- to 20-fold increased risk of amputation[1] and have a one-year mortality rate as high as 5%[14]. As many as 20% of DFUs remain unhealed after one year of treatment, with unhealed ulcers posing a risk for infections, gangrene, amputation, and even death[15, 16].

There is a strong association between the development of DFUs and DR (Figure 2)[5]. Depending on the population studied, most individuals affected by DFUs also have DR[6,17-19], and those with DR are two to four times more likely to have DFUs or more serious forms of diabetic foot disease (Table 1)[20-22]. Even more concerning is the strong association between DFUs and proliferative diabetic retinopathy (PDR), with 31% to 55% of individuals having this more severe stage of DR (see below)[23]. Furthermore, patients with nonproliferative diabetic retinopathy (NPDR) who develop comorbid non-

Table 1 Studies examining association of diabetic foot disease and diabetic retinopathy

Ref.	Year	Type of study	Sample size (DFU; no DFU)	Source of population	Main findings
Jayaprakash *et al*[17]	2009	Prospective Case Study	94	India	73.4% prevalence of DR in patients with DFUs
Hwang *et al*[22]	2017	Retrospective Cohort	100	South Korea	90% prevalence of DR in patients with type 2 DM and DFUs; 55% had PDR
Karam *et al*[18]	2018	Cross-sectional	182	India	67.6% prevalence of DR in patients diabetic foot disease (including neuropathy, deformation, DFUs, or amputation)
Zafar *et al*[19]	2019	Cross-sectional	530 (225; 305)	Pakistan	96% of patients with DFUs had DR
Sellman *et al*[23]	2020	Case Control	270 (90; 180)	Sweden	31% prevalence of PDR in patients with DFUs
Banik *et al*[21]	2020	Cross-sectional	680 (8; 672)	Bangladesh	65.9% prevalence of DR in patients with DFUs
Ye *et al*[20]	2014	Retrospective Cohort	829 (61; 768)	China	OR 2.026 for DFUs in patients with DR
Al-Rubeaan *et al*[6]	2015	Retrospective Cohort	62681 (2071; 60610)	Saudi Arabia	OR 4.45 for diabetic foot disease (including DFUs, gangrene, and amputation) in patients with DR
Harris Nwanyanwu *et al*[24]	2013	Retrospective Cohort	4617	United States	1.54 HR for those with comorbid non-healing DFUs to progress from NPDR to PDR in three to five years

DR: Diabetic retinopathy; NPDR: Nonproliferative diabetic retinopathy; PDR: Proliferative diabetic retinopathy; DFU: Diabetic foot ulcer; OR: Odds ratio; HR: Hazard ratios.

healing DFUs have a greater than 50% increased risk of progressing to PDR relative to those without this condition[24]. Finally, diabetic keratopathy is an important ocular parallel of DFUs. It is a disruption of normal corneal wound healing and loss of protective mechanisms of corneal sensation and aqueous tear production. These aberrations create an ideal environment for persistent corneal epithelial defects, microbial infection, and ulceration. Around half of patients with DM are affected by this condition[25]. The pathophysiology accounting for diabetic structural and functional alterations in the cornea is discussed in depth below.

Microvascular complications

Microvascular dysfunction in the lower extremity contributes to impaired function and impedes wound healing, which promotes the development of DFUs. Damage to endothelial cells from chronic hyperglycemia, oxidative stress-induced injury, generation of advanced glycation end-products, increased polyol flux regulated by aldose reductase, activation of protein kinase C (PKC), and other pro-inflammatory processes from immune dysregulation cumulatively disrupt normal blood flow and affect vascular permeability[26]. In its most severe form, this compromise of the microvasculature leads to ischemia and a relative hypoxic state in the involved tissue. As a result, there is increased expression of hypoxia-inducible factor-1 (HIF-1) leading to the production of vascular endothelial growth factor (VEGF), a protein principally responsible for restorative angiogenesis[27]. However, in DM there are disturbances in cytokine growth factor expression and locally decreased concentrations of VEGF, which render the lower extremity vulnerable to poor wound healing[9]. Failure of the microvasculature also contributes to peripheral neuropathy and local immune dysfunction, including impaired cellular response, cytokine expression, and vascular tone[28].

In the eye, these same pathways driven by hyperglycemia and other diabetes-associated factors lead to progressive damage to the retinal microvasculature and cause the development of DR[29]. DR develops in roughly one quarter of patients with DM, with the prevalence being highest in Africa (36%) and lowest in South and Central America (13%)[3,30]. Initially, the disease manifests as clinically detectable changes in the retinal vasculature, including the development of microaneurysms and loss of capillaries which are the hallmarks of early NPDR[31]. As the disease progresses, the production of VEGF and other diabetes-associated factors promotes further dysfunction, vascular leakage, and bleeding (dot-blot hemorrhages)[32]. At this stage, visual acuity is increasingly likely to be affected and is often further limited by swelling in the center of the retina, known as diabetic macular edema (DME)[33]. The development of neovascularization on the optic nerve or at locations in the peripheral retina signifies the progression to PDR. This is the most vision-threatening complications of the disease also primarily driven by the abnormal expression of VEGF[29,30,34]. These fragile new vessels, which grow into the vitreous cavity and along the inner retinal surface, often bleed, causing vitreous hemorrhages, traction, or retinal detachment and thereby impair vision[3,32]. Finally, excessive expression of VEGF may also affect the anterior segment of the eye by causing neovascularization on the iris and ciliary body. When the growth of this fibrovascular tissue extends to the anterior chamber angle it may block

outflow of aqueous humor through the trabecular meshwork causing eye pressure to rise to levels capable of damaging the optic nerve in a disease process known as neovascular glaucoma[4,32]. When left unaddressed, irreversible blindness results.

Clinical examination supplemented by diagnostic color fundus photography and fluorescein angiography are the mainstays for staging DR; however, emerging modalities including ultrawide-angle imaging and optical coherence tomography and angiography increasingly allow clinicians to directly and noninvasively visualize the diseased retina and its microvasculature[31]. Advances in therapeutic modalities, such as intraocular injection of agents that target VEGF, steroids that target inflammation, and panretinal laser photocoagulation, have improved clinical outcomes for patients with DR[33-36]. However, effective long-term management is largely dependent upon regular follow-up care. Patients who fail to return for care are more likely to suffer vision loss[37,38].

The intraocular administration of agents that target VEGF are now the most common treatments for DR and DME[29,36]. Thankfully these agents are very unlikely to negatively impact wound healing in the lower extremity, especially at the doses employed to treat eye disease[39]. Similarly, intraocular corticosteroids, such as dexamethasone, and intravitreal steroid implants utilized to treat DME have been found to have no detectable influence on HbA1c or renal function[40,41]. However, when larger doses are administered as subconjunctival or peribulbar injections, some patients can experience elevations of blood glucose, similar to that observed with oral and intravenous administration of corticosteroids[42]. Finally, topical steroid drops have only very rarely been associated with endocrinological side effects in case reports[43].

Wound healing

Wound healing in the lower extremity requires coordinated cellular responses that cause an organized release of growth factors and cytokines. Under normal conditions, when an injury occurs, multiple cell types, including macrophages, fibroblasts, and epithelial cells, release VEGF and other cytokines in response to local ischemia caused by the wound[44]. However, in patients with DM, disturbances in cytokine and growth factor expression, including fibroblast growth factor, insulin-like growth factor, platelet derived growth factor, and VEGF, among others, lead to a condition that subsequently permits prolonged hypoxia[9,45]. Additionally, keratinocytes and fibroblasts in DFUs have demonstrated attenuated cellular migration, proliferation, and protein synthesis, resulting in impaired re-epithelialization which further exacerbates the oxygen-restricted wound[46]. Moreover, hyperglycemic states reduce the stability and function of HIF-1, which further impairs the wound healing response as a downstream consequence of sustained hypoxia[9]. Increased free radical damage is also a known causative factor in impaired wound healing in patients with DM. Inappropriately elevated concentrations of reactive oxygen species (ROS) and impaired antioxidant enzyme activity can cause nerve damage and directly contribute to the progression of peripheral neuropathy[47].

Individuals with DM also have abnormal wound healing pathways in the eye. Notably, corneal thinning is thought to be the earliest detectable pathological manifestation of DM in the eye[48]. Diabetic keratopathy leads to persistent corneal epithelial defects and neurotrophic corneal ulcers that respond poorly to treatments applied in the hyperglycemic environment[49]. Abnormalities in corneal cell morphology, varied number and disorganization of epithelial cell layers, impaired cellular migration, reduced endothelial cell number, and accumulation of acellular debris all contribute to poor wound healing[50]. Sectorial thinning, bullae, and persistent corneal epithelial defects from diabetic keratopathy often lead to corneal ulcers, scarring, and reactive neovascularization, which cause decreased visual acuity or permanent vision loss[50,51]. Although the cornea itself is avascular and ischemia does not play a significant role in diabetic keratopathy, wound healing in the cornea, like in the lower extremity, requires highly structured cellular processes which are impacted by hyperglycemia. These involve proliferation and migration of epithelial cells, fibroblasts, and the expression of numerous growth factors, including transforming growth factor beta, epidermal growth factor, insulin-like growth factor, and platelet derived growth factor[51]. Finally, diabetes-associated hyperglycemia may also impair vision by accelerating the progression of diabetic cataract and impact the health of the lens epithelium[52].

Most treatments for diabetic foot and eye problems are applied locally, but some treatments to aid the lower extremity may have theoretical consequences on the eye, and vice versa. Several adjuvant therapies have been found to reduce DFU healing times and amputation rates, including non-surgical debridement agents, topical dressings and agents, negative pressure wound therapy, oxygen therapies, acellular bioproducts, and human growth factors[53]. Oxygen is required for almost every step of the wound healing, affecting cell proliferation, collagen synthesis, and re-epithelialization, as well as immunologic defense against bacteria and other pathogens[54]. Oxygen may be delivered in the form of local, hyperbaric, or supplemental inspired oxygen therapy. Hyperbaric oxygen therapy has proven to be particularly useful in managing chronic, non-healing DFUs, especially in the relatively ischemic diabetic foot, albeit at a high financial cost[55]. Patients have been observed to have increased tissue concentrations of VEGF after completing hyperbaric therapy sessions; this has been attributed to the sharp decline of relative oxygen concentration once a session is completed[56]. As previously mentioned, the presence of VEGF is the primary driver of DR, so there is a theoretical risk that systemic or local oxygen therapy could exacerbate this condition. However, empiric evidence does not suggest

that oxygen therapy is harmful to the diabetic eye[23]. It has even been reported that patients with concurrent DR have benefitted from the administration of hyperbaric oxygen therapy through supranormal levels of oxygen delivered to the retina[57]. Nevertheless, it remains essential that the status of DR is assessed and regularly monitored in any patient undergoing oxygen therapy for DFUs.

Many growth factors that have been identified as integral to wound healing are also potential therapeutic targets. In the diabetic foot, among the most promising are hydrogels which contain recombinant PDGF, approved by the United States Food and Drug Administration for topical administration having demonstrated improved rates of DFU healing in randomized clinical trials[58]. In the diabetic cornea, recombinant human nerve growth factor (NGF), epidermal growth factor, and metalloprotease inhibitors have demonstrated some success in trials for the treatment of diabetic keratopathy [59]. Of note, the opioid antagonist naltrexone has been demonstrated to improve wound healing, corneal surface sensitivity, and tear secretion in diabetic animal models[60,61]. The future will also likely include gene- and cell-based therapies to accelerate wound healing, including in DFUs and diabetic cornea[62,63].

Diabetic neuropathy

Approximately half of adults with DM will be affected by peripheral neuropathy in their lifetime[64]. Peripheral neuropathy typically begins with diminution or loss of protective sensation. In addition, loss of proprioception contributes to injuries and falls[11]. Moreover, autonomic dysregulation in the foot may contribute to impaired cutaneous blood flow, sweating dysfunction, and loss of vascular tone that compromise integument integrity and wound healing[65]. Lower extremity deformities may also occur, such as hammer toes or claw toes, which are associated with loss of function[11]. Finally, delays in the identification of accidental and iatrogenic injuries because of reduced sensation may cause patients to fail to seek care in a timely fashion and increase the risk for infections[64].

As mentioned above, chronic hyperglycemia from DM causes microangiopathic changes. In the case of diabetic neuropathy, hyperglycemia may affect the endoneurial microvasculature by directly reducing perfusion and impairing nerve function[66]. Many of the same cellular and biochemical mechanisms linked to chronic hyperglycemia injure the peripheral nerves, including increased glycolytic processes producing oxidative stress, generation of advanced glycation end-products, increased polyol flux regulated by aldose reductase, PKC activation, and other pro-inflammatory processes from immune dysregulation[67]. Damage to mitochondria also plays an important role in the pathogenesis of diabetic neuropathy and contributes to nerve dysfunction, cell death, and loss of neurotrophic support provided by neurotrophin-3 and NGF[68]. Patients who develop PAD also have more severe diabetic neuropathy (see below)[64].

The cornea is the most densely innervated tissue of the human body and is 100 times more sensitive than skin[69], but this declines with age[70] and is further reduced by DM[71]. The loss of protective sensation of the diabetic cornea impacts various homeostatic functions, such as blinking, aqueous tear production, and the release of growth factors[51,52]. As a result, the incidence of dry eye disease and the need for artificial tears is increased among patients with DM, particularly among those with worse diabetes-related outcomes[72]. A recent meta-analysis estimated that DM conferred 30% increased odds for dry eye syndrome[58]. Dry eye disease and DR are also associated with each other[73]. These changes result in neurotrophic keratopathy marked by persistent epithelial defects and chronic erosions that may develop into corneal ulcers, corneal scarring, and neovascularization, all of which contribute to visual dysfunction[74] and predispose patients to infectious keratitis[75]. The recent application of *in vivo* confocal microscopy has allowed for visualization of diabetes-associated structural changes in the nerves of the corneal epithelium, including nerve thickening[76] and decreased nerve length and density[77]. Anterior segment optical coherence tomography is another emerging diagnostic modality used to evaluate and manage diabetic keratopathy by enabling the direct visualization of the cornea structure and nerves[50,71].

Diabetes-associated hyperglycemia has also been shown to cause direct injury to the neuronal retina, leading to thinning of the nerve fiber layer from the loss of ganglion cells and the death of other retinal neurons, including photoreceptors[78]. This may lead to decreased visual function, impaired contrast sensitivity, and diminished night vision[29,32]. Finally, the eye may also be suddenly and directly affected by diabetic cranial neuropathies, manifesting as double vision from ophthalmoplegia, which is the paralysis of the muscles that move the eye (see below).

Current recommendations for the management of painful diabetic neuropathy include gabapentinoids, serotonin and norepinephrine reuptake inhibitors (SNRIs), and tricyclic antidepressants (TCAs)[79]. Gabapentin is a well-tolerated medication from an ophthalmic standpoint; its most common adverse effect is a reversible nystagmus[80]. SNRIs, such as venlafaxine, have been associated with acute angle closure glaucoma in some case reports[81], along with increased cataract development[82]. Finally, TCAs are associated with blurred vision in up to one third of patients, likely due to the anticholinergic action of these drugs[83]. These side effects further emphasize the importance of communication and collaboration with ophthalmologists when treating diabetes-associated complications of the lower extremity.

Macrovascular complications

Common macrovascular complications of DM that affect the lower extremity include PAD and chronic venous insufficiency (CVI), which may lead to lower extremity amputation (Figure 1)[84]. DM induces and accelerates the development of atherosclerosis *via* multiple mechanisms that include metabolic derangements, smooth muscle dysfunction, oxidative stress, potentiated platelet function, increased coagulability, and chronic inflammation[85]. The availability of the potent vasodilator nitrous oxide, which is produced in the endothelium and is a primary mediator in local vascular endothelial tone, is reduced in hyperglycemic states; DM also promotes the production of endothelin-1, which indirectly increases vasoconstriction and vascular smooth muscle hypertrophy[86]. The result may be an overt occlusion, sometimes acutely when a thrombus forms, or when increasingly stenotic vessels result in reduced perfusion[86]. Patients with co-existing severe PAD are also more likely to have CVI[84], which contributes to poor wound healing by increasing hydrostatic pressure in the lower extremity, thereby promoting wound exudation[87].

While not directly a macrovascular complication, it is important to recognize that DR is strongly associated with lower-extremity PAD. Patients with DR have an approximately two-fold increase in the need for lower-limb revascularization and a five-fold increase in lower-limb amputation[88]. Patients with PAD benefit from additional medical management and risk factor modification for atherosclerotic disease. In addition to optimizing diabetes control, this includes counseling about smoking cessation, antiplatelet and statin therapies, as well as blood pressure control[89]. Exercise also plays a fundamental role in the treatment of PAD, leading to reductions in pain and improvement in functional capacity[89]. The clinical benefit of newer medications on amputation prevention remains uncertain.

In the eye, DM-associated macrovascular disease can manifest as an ocular ischemic syndrome (OIS), a rare, but vision-threatening condition associated with severe carotid artery occlusive disease that leads to ocular hypoperfusion[90]. Like PAD, atherosclerosis affecting the vessels supplying the eye is the main cause of the disease, and most patients with OIS have a diagnosis of DM[91]. Patients typically report dull eye or periorbital pain associated with gradual vision loss as the retina experiences progressive ischemia. Consequently, VEGF levels rise, which may cause neovascular glaucoma in the anterior segment and reduce the final visual potential; neovascularization can also develop in the retina, but it is less prominent than in DR because of reduced retinal perfusion[92]. OIS entails an overall poor visual prognosis, which means that the ophthalmologist's diagnosis is crucial for the systemic health of those patients because OIS may be the presenting sign of impending serious cerebrovascular and ischemic heart disease. Finally, DM can sometimes cause an ischemic optic neuropathy, which is a direct infarct of the optic nerve[52].

Another condition involving a main function of the eye where macrovascular disease in DM manifests is ophthalmoplegia. Ophthalmoplegia is the paralysis of one or more of the extraocular muscles (EOM). It can arise from traumatic, autoimmune, infectious, and vascular etiologies. Usually involving the third (oculomotor), fourth (trochlear), or sixth (abducens) cranial nerves, double vision is the characteristic symptom of ophthalmoplegia[93]. The vascular supply for the EOMs comes from branches of the ophthalmic artery, which is itself a branch of the internal carotid artery. Additionally, the cranial nerves responsible for the EOMs themselves have a complex vascular supply. Focal cranial nerve ischemia due to atherosclerosis within the microvasculature is thought to contribute to the development of ophthalmoplegia in patients with DM[94].

PREVENTION AND MANAGEMENT

Preventing diabetic foot and eye problems is best achieved through regular examinations, diabetes education, and optimal management of underlying DM and its associated metabolic consequences. Tight control of blood glucose, as reflected by HbA1c level, is the most important element for prevention of these two interrelated diabetes-associated complications, closely followed by optimization of blood pressure and lipid levels[1,7,37,38]. This is accomplished through a combination of regulation of diet, lifestyle modification, body mass reduction, and medications, such as insulin and/or oral antidiabetic therapies, as appropriate. Monitoring alterations in serum biomarkers, such as HbA1c, ceruloplasmin, creatinine, uric acid, LDL, and HDL, is also important because these biomarkers are associated with both the onset and severity of DR and DFUs[8,20].

The standard practices in DFU management include cleansing, surgical debridement, application of clean dressings to maintain a moist environment and control exudates, wound off-loading, vascular optimization (including revascularization procedures), treatment and prevention of infection, and glycemic control[88,95]. Proper instruction is also required to prevent accidental or iatrogenic injuries which can result from ordinary hygiene and grooming of the feet and lower extremity[29]. Infection prevention is best achieved through protective footwear, proper hygiene, and offloading interventions [11]. Similarly, preventing complications from diabetic keratopathy focuses on limiting repetitive trauma, neurosensory deformities, exposure, and infections. Injuries may be caused by eye droppers, abnormal eye lashes, cosmetic applicators, fingers, facial towels, and bedding[96]. Infection can occur from overgrowth of the ocular flora or opportunistic infection enhanced by hyperglycemia, or it can

take the form of chronic and recurrent herpes simplex and zoster[97].

Many parallels exist between management of ulcers in the cornea and those in the lower extremity. Both are treated with clean dressings, antimicrobial ointments, and salves. Wound infections may be polymicrobial, but the bacterial species most associated with DFUs include gram-positive species, *e.g.*, Staphylococcus aureus and Streptococcus species, but gram-negative infections with Pseudomonas aeruginosa and Enterobacteriaceae species also occur and are notably more common in ischemic or deep wounds[98]. Infection of corneal ulcers involve many of these same organisms, including Staphylococcus, Pseudomonas aeruginosa, and Streptococcus pneumoniae[70,99]. Special dressing and vacuum-assisted wound closure have been used with good result in the management of DFUs[100]. Non-healing diabetic corneal ulcers are often treated in conjunction with bandage contact lenses, which can lengthen the time antibiotic treatments are in contact with the ocular surface and serve as a reservoir for pharmacologically active compounds to aid wound healing[69]. In contrast to DFU management, patching should generally be avoided in patients with DM and corneal disease because of an increased risk of infection[101]. Amniotic membrane grafts have been studied for their potential of facilitating epithelial migration and healing of corneal ulcers and in very severe cases, corneal transplantation may be necessary[102].

Emerging research has placed an emphasis on developing therapeutic options that offer additional ways of preventing diabetic complications, treating them at earlier stages, or in more effective ways. As discussed earlier, inflammation has been implicated in the pathogenesis of diabetes-associated complications. Cytokines, such as interferon-γ, are being investigated as potential therapeutic targets in attenuating inflammatory cascades given that many of these cytokines contribute to altered vascular permeability and angiogenesis[103]. Given the high metabolic rate of the retina in conjunction with the metabolic stress induced by chronic hyperglycemia, reducing free radical stress may be an effective strategy[104]. Polyphenols, such as epigallocatechin-3-gallate found in green tea, are known for their antioxidant and anti-inflammatory properties and in diabetic animal models, have been shown to attenuate ROS concentrations in the retina[105]. Other polyphenol compounds, carotenoids, thiols, and vitamin supplementation are being investigated to address the several pathways involved in ROS generation and inflammation[104].

Finally, a multidisciplinary care team is essential to care optimally for the diabetic foot and eye, preserving function and quality of life for those with DM (Figure 3). Primary care providers and endocrinologists play a crucial role in coordinating care, including providing a formal assessment of the degree of diabetic control, screening for symptoms related to diabetic complications affecting other organ systems such as diabetic nephropathy, prescribing DM treatment, and involving specialists who manage diabetic complications such as foot or eye problems[1]. A diabetes care team should also include pharmacists who provide medication therapy management, dieticians, psychologists, diabetes care managers, and nurse educators. By working together, a coordinated care team can effectively reduce the healthcare burden associated with DM and its complications through prevention, screening, and management. In the future, the integration of smartphone technology and telehealth may not only streamline care coordination, but also allow for remote diagnosis and long-term monitoring of disease [106].

CONCLUSION

The identification of any ophthalmic or lower extremity complication in a patient with type 1 or type 2 DM should immediately prompt a review of DM management and coordination of diabetes care, including referral for reciprocal comprehensive foot or eye evaluations in patients with either complication[1,37,38]. Although diabetic foot disease is slightly more common among patients with type 1 DM and those who use insulin[6], optimizing diabetes management remains the most important step in preventing diabetes-associated complications no matter what the type of DM[37,38]. While many patients may report symptoms related to diabetic foot disease or observe vision loss in the setting of diabetic eye problems, many others may be asymptomatic or have such mild signs and symptoms that they are easily overlooked, dismissed, or fail to receive clinical attention unless specifically assessed[64,107]. Primary care providers and endocrinologists should perform regular diabetic foot examinations because they provide insight into the presence and degree of PVD, neuropathy, skin breakdown, and other pre-ulcerative changes. Providers must also screen for signs and symptoms of eye disease, in part because their identification may help triage the urgency of any necessary referrals[37,38]. Because diabetic eye and foot diseases so commonly occur in conjunction, it is essential that clinicians take the necessary steps to reduce the impact of these diseases through regular screening, prompt referral to specialists, and providing a coordinated, team-based approach to management.

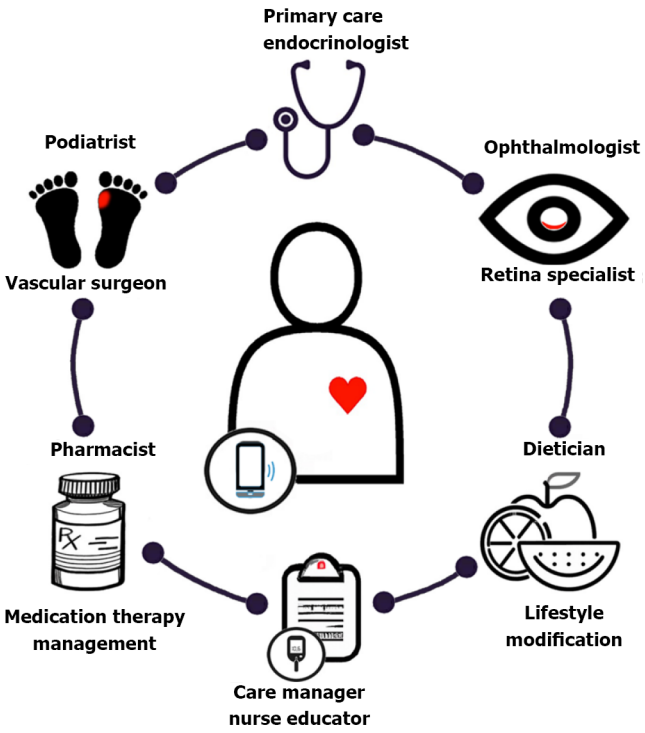

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Figure 3 Diabetic care team process. Primary care and endocrinology physicians are central to comprehensive diabetes mellitus (DM) evaluation including assessment of the level of glycemic control, prescription of medications, determining level of treatment adherence, and identification of gaps in care and risk of complications. The frequent concurrence of diabetic eye and foot problems mandate that patients affected by either condition should undergo reciprocal comprehensive eye and foot evaluations, in addition to optimizing diabetic control. Specialists are often required to manage diabetic foot problems, including referral to podiatry, lower extremity wound care specialists, or vascular surgery, each engaging treatment algorithms according to their expertise. Eye care is typically provided by ophthalmologists or optometrists, but often requires the expertise of a retinal specialist capable of providing the medical and surgical management of diabetic eye disease. Pharmacists provide medication therapy management and are an important sources of diabetes education. Dietitians, lifestyle coaches, and psychologists offer counseling that works toward improving or maintaining glycemic targets through nutrition, achieving weight management and physical activity goals, and implementing behavior changes. Diabetic care managers and nurse educators help individuals with DM establish long-term commitments. They provide instruction on foot and skin care; the use of medications, including the administration of insulin; the monitoring of blood glucose levels; and maintenance of proper diet and exercise. They develop an overall management strategy aimed at reducing risk factors linked to diabetes-associated complications. The integration of smartphone technology and telehealth may streamline the care coordination and communication between the patient and each component of the diabetic care team [106]. Figure 3 was made in ©BioRender-biorender.com. The authors generated parts of the digital images used in Figure 3 by using the Generative Pre-trained Transformer 3 (GPT-3) autoregressive language model that employs deep learning to generate digital images from natural language descriptions (DALL·E, OpenAI, San Francisco, CA, labs.openai.com). The authors reviewed, edited, and revised these images and take ultimate responsibility for the content included in this publication.

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FOOTNOTES

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REVIEW

Diabetic foot ulcers: Classification, risk factors and management

Xuan Wang, Chong-Xi Yuan, Bin Xu, Zhi Yu

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Abstract

Diabetic foot ulceration is a devastating complication of diabetes that is associated with infection, amputation, and death, and is affecting increasing numbers of patients with diabetes mellitus. The pathogenesis of foot ulcers is complex, and different factors play major roles in different stages. The refractory nature of foot ulcer is reflected in that even after healing there is still a high recurrence rate and amputation rate, which means that management and nursing plans need to be considered carefully. The importance of establishment of measures for prevention and management of DFU has been emphasized. Therefore, a validated and appropriate DFU classification matching the progression is necessary for clinical diagnosis and management. In the first part of this review, we list several commonly used classification systems and describe their application conditions, scope, strengths, and limitations; in the second part, we briefly introduce the common risk factors for DFU, such as neuropathy, peripheral artery disease, foot deformities, diabetes complications, and obesity. Focusing on the relationship between the risk factors and DFU progression may facilitate prevention and timely management; in the last part, we emphasize the importance of preventive education, characterize several of the most frequently used management approaches, including glycemic control, exercise, offloading, and infection control, and call for taking into account and weighing the quality of life during the formulation of treatment plans. Multidisciplinary intervention and management of diabetic foot ulcers (DFUs) based on the effective and systematic combination of these three components will contribute to the prevention and treatment of DFUs, and improve their prognosis.

Key Words: Diabetes; Diabetes foot ulceration; Classification; Diabetes complications; Clinical management; Lower limb complications

Core Tip: Diabetic foot ulcers (DFUs) are a common complication of diabetes. The high recurrence and amputation rates associated with DFUs reflect an urgent need to improve care and treatment methods, highlighting the importance of a comprehensive investigation of the important components of clinical diagnosis and treatment. This article reviews the classification and risk factors of DFUs and summarizes the common clinical management approaches.

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INTRODUCTION

The prevalence of diabetes mellitus (DM) is rapidly spreading at an alarming rate worldwide[1]. DM is known to damage multiple organs, including the heart, kidney, eye, and nerves, leading to complications such as heart attack, stroke, blindness, kidney failure, and lower limb amputation. Diabetic foot ulcer (DFU) is a frequent complication that occurs in approximately 6.3% of patients with DM globally [2]. The high incidence of DFU and the associated mortality and morbidity are the most common reasons for hospitalization of diabetes patients. Early in the course of DM, patients experience serious foot sensitivity symptoms such as pain and tingling, while later stages of the disease course are characterized by negative symptoms such as numbness and weakness of the toes. With the progression of the disease, patients usually show mixed pain sensitivity and dullness, along with decreased limb sensation and motor function, which lead to imbalance and unsteadiness and increase the likelihood of falls[3,4]. In addition, because of the increasing morbidity, DFU is a leading cause of non-traumatic amputation and is associated with an increased risk of death[5].

The high incidence and intractability of DFU extract a substantial cost in terms of reduced productivity and increased healthcare-related expenses. Appropriate and prompt treatment of DFU requires a multifaceted approach, including timely and correct diagnosis and classification, multiple assessments of risk factors, and appropriate choice of management, all of which should be based on the patient's actual condition. This primer will present the current knowledge of the potential pathogenesis of DFU, discuss the clinical classification of DFU, highlight the corresponding approaches to diagnosis and common management techniques, and close with a call that more attention and feasible interventions are required for DFU management.

DEFINITION AND CLASSIFICATION OF DFU

Definition

The practical guidelines formulated by the International Working Group on the Diabetic Foot (IWGDF) defined DFU as a set of symptoms secondary to current or previous diabetes, including skin chapping, ulceration, infection, or destruction of foot tissue, which partly reflects the fuzzy and imprecise nature of this concept[6,7]. DFU is a complicated and multifactorial clinical problem that affects many patients with diabetes, who experience ulceration and infection, invariably with neuropathy and/or peripheral artery disease (PAD), that disrupt the foot epidermis and dermis, breach the skin envelope, expose sterile structures, and finally form full-thickness lesions[8]. In the Western world, more than 60% of non-traumatic amputations involve DFU, which leads to an increase in hospitalization rate and mortality[9] and causes reduced quality of life (QoL). Moreover, treatments based on amputation impose a heavy burden on the economic and health resources of patients with diabetes[10].

Classification

The multiple factors associated with the development of DFU, such as the complex process and complications of diabetes, may all lead to various degrees of neurological abnormalities and vascular damage (known as neuropathy and PAD)[11]. Once the ulcer is formed, the factors affecting healing may be more complex, and different factors may dominate at different stages over time. Thus, these related factors play different roles depending on the severity of disease and duration of recovery, necessitating different diagnoses and treatments for seemingly the same symptoms and causing differences in the curative effect[12]. In these circumstances, the classification and scoring criteria for describing lesions of DFU should be formatted in a manner that is clinically recognized and widely used, which will allow characterization of DFU on the basis of differences and facilitate suggestions for treatment or care programs.

Considering the different audiences and objectives of the classification and scoring systems, no universally accepted system has been published to date. Various systems are used to describe and assess the severity of DFU, and three types of key factors contributing to the scoring system have been proposed, namely, patient-related, limb-related, and ulcer-related factors, which reflect end-stage renal failure, PAD, and loss of protective sensation, along with classification of the wound grade[13]. Most systems set scoring criteria based on the size and characteristics of the wound, such as size, depth, ischemia, and infection, allowing characterization of the lesion, while risk factors such as neuropathy and peripheral arterial occlusive disease are incorporated when clinical interventions or preventive guidance are required[14,15]. In this section, we will introduce several major systems and summarize their characteristics and applications.

The Meggitt-Wagner system: This system, which was described by Meggitt in 1976 and disseminated by Wagner in 1979, was once the most widely used system[16-18]. It is a six-grade classification system mainly covering the depth of the ulcer and the degree of tissue necrosis[19] (Table 1). This system, which is essentially wound-based, is intuitive and simple to use, but since it does not consider clinical parameters such as peripheral neuropathy and PAD, it cannot distinguish between infection and ischemic lesions, which is also related to its recognized imprecision and limitations[20].

The University of Texas classification system: The classification system proposed by the University of Texas (UT) takes some common clinical signals and symptoms of DFU into consideration by using a 4 × 4 matrix assessing ulcer depth horizontally and infection and ischemia status vertically[15,21] (Table 2). Since it aims to divide patients into four categories depending on whether they are infected or ischemic on the premise of distinguishing the depth of ulcer, the UT system is more helpful to predict amputation than the Meggitt-Wagner system, which simply classifies the ulcer condition[21,22].

The size (area, depth), sepsis, arteriopathy, denervation system: The size (area, depth), sepsis, arteriopathy, and denervation [S(AD)SAD] system was proposed in 1999 and is mainly designed for clinical audits[23]. The system was first verified in 2004, and in order to further refine the classification of ulcers for prospective research, some criteria missing from the UT system were included subsequently[24]. This system contains five elements that are scored in grades 0-3 according to severity, namely, size (area, depth), infection (sepsis), ischemia (arteriopathy), and neuropathy (denervation), and uses acronyms to facilitate memorization and feature generalization[24] (Table 3). The advantage of this system lies in its ability to allow specific recording of ulcers without requiring professional testing technology and equipment, facilitating its usage in clinics. However, because of the multiple descriptions of characteristics and irregular details of ulcers, the system is difficult to remember for operators, which may be the reason why the S(AD)SAD system is considered to be more suitable for audits while the UT system is used for clinical description and communication[25,26].

The site, ischemia, neuropathy, bacterial infection, area, depth system: A simplified and refined form of the S(AD)SAD system, the site, ischemia, neuropathy, bacterial infection, area, depth (SINBAD) system, was proposed to reduce the difficulties in clinical use caused by the inclusion of more complicating criteria while retaining the descriptions of ulcer characteristics to the maximal extent possible[12,27]. The SINBAD system still contains five elements (area, depth, infection, ischemia, and neuropathy), and grades each element as either 0 or 1 point to create an evaluation system with scores of 0-6 for description of increasing severity[27] (Table 4). The modified system is simple but sufficiently robust and allows collection of the necessary information without specialist equipment, except for routine clinical examinations[13]. It has been proven to have moderate inter-observer and excellent intra-observer reproducibility and may help accurately describe the progress of ulcers, including healing and the need for amputation, which was confirmed by the fact that IWGDF recommends the SINBAD system[28].

The Wound, Ischemia, and foot Infection system: Because of the rising prevalence of neuroischemic ulcers, the dichotomy for ischemia in the existing systems lacks effective severity grading and cannot meet clinical requirements. In 2014, the Wound, Ischemia, and foot Infection (WIfI) system was proposed by the Society for Vascular Surgery Lower Extremity Guidelines Committee, and it covered the three most important risk factors that may cause amputation of lower limbs: WIfI[29]. The three factors are assigned scores from 0 to 3, of which the wound is graded on the basis of size, depth, severity, and anticipated difficulty in achieving wound healing; ischemia is rated on the basis of ABI gradation; and foot infection is rated on the basis of the scope and depth of the wound[29] (Table 5). Clinical studies have suggested that this system primarily offers value in predicting major amputation [30]. In patients with DFU and vascular disease, the WIfI system is recommended to evaluate perfusion and vascular function and help rapidly implement revascularization and/or drainage[31]. Since the evaluation of foot perfusion indices requires specialist measurements, assessments using this system require expertise in vascular intervention, indicating that it is not ideal for use in primary and/or community care[13].

Table 1 Wagner classification system

Grade	Ulcer depth
0	Pre-ulcerative area without open lesion
1	Superficial ulcer (partial/full thickness)
2	Ulcer creep to tendon, capsule, bone
3	Stage 2 with abscess, osteomyelitis, or joint sepsis
4	Localized gangrene
5	Global foot gangrene

Table 2 University of Texas classification system[21]

	Grade 0	Grade 1	Grade 2	Grade 3
	Pre- or post-ulcerative site	Superficial wound not involving tendon, capsule, or bone	Wound penetrating to tendon or capsule	Ulcer penetrating to bone of joint
Lesions without infection or ischemia				
Infected/non-ischemic lesions				
Ischemic noninfected lesions				
Ischemic infected lesions				

Table 3 Size (area, depth), sepsis, arteriopathy, denervation system

Grade	Size				
	Area	Depth	Sepsis	Arteriopathy	Denervation
0	Skin intact	Skin intact	None	Pedal pulses present	Pin pricks intact
1	< 1 cm^2	Superficial (skin and subcutaneous tissue)	Surface	Pedal pulses reduced or one missing	Pin pricks reduced
2	1-3 cm^2	Tendon, periosteum, joint capsule	Cellulitis	Absence of both pedal pulses	Pin pricks absent
3	> 3 cm^2	Bone or joint space	Osteomyelitis	Gangrene	Charcot

RISK FACTORS FOR DFU

DFU is caused by multiple interacting risk factors, of which the most common major identified factors include diabetic neuropathy (DPN), PAD, and foot deformities. These factors can be further divided into different degrees according to the severity[32-36]. In this section, the main risk factors are listed and introduced.

Neuropathy

The neuropathy induced by diabetes is a symmetric polyneuropathy that affects the sensory, motor, and autonomic components of the peripheral nerves to varying degrees[37]. Epidemiological data shows that neuropathy is responsible for 16%-66% of the cases of diabetic foot syndrome[38], and patients with neuropathy are prone to show relapse after healing, eventually leading to lower limb amputation[39]. DPN results in the loss of protective sensation, usually starting in a symmetrical and sock-like manner. Small and unmyelinated nerve fibers responsible for conducting afferent sensory perception, like C-type fibers, are the first to be damaged, resulting in tissue damage due to poor perception of trauma and/or mechanical stress. Thus, the relatively minor damage will continue to accumulate and result in a progressively worsening wound with difficulty in healing[33].

Motor neuropathy causes atrophy of foot muscles by denervation of specific muscle groups, which directly affect the function of the foot. Since the small muscles of the foot, like the extensor digitorum brevis and lumbrical and interosseous muscles, are paralyzed gradually, the anatomy of the foot arch changes, and the metatarsophalangeal joints (MTPJs) become hyperextended or over-contracted[40,41]. The joints remain movable in the initial stage, but with aggravation of the symptoms, the

Table 4 Site, ischemia, neuropathy, bacterial infection, area, depth system[13]

Category	Definition	Score
Site	Forefoot	0
	Midfoot and hindfoot	1
Ischemia	Pedal blood flow intact: At least one palpable pulse	0
	Clinical evidence of reduced pedal flow	1
Neuropathy	Protective sensation intact	0
	Protective sensation lost	1
Bacterial infection	None	0
	Present	1
Area	Ulcer < 1 cm^2	0
	Ulcer ≥ 1 cm^2	1
Depth	Ulcer confined to skin and subcutaneous tissue	0
	Ulcer reaching muscle, tendon or deeper	1
Total possible score		6

Table 5 Wound, Ischemia, and foot Infection system

Grade	Wound	Ischemia			Foot infection system
	Clinical features	ABI (mmHg)	ASP (mmHg)	Toe pressure, $TcPO_2$ (mmHg)	Clinical manifestations
0	No ulcer no gangrene	≥ 0.80	> 100	≥ 60	No symptoms or signs of infection. Infection present, as defined by the presence of at least two of the following items: (1) Local swelling or induration; (2) Erythema 0.5 cm-2 cm around the ulcer; (3) Local tenderness or pain; (4) Local warmth; and (5) Purulent discharge (thick, opaque to white, or sanguineous secretion)
1	Small, shallow ulcer(s) on the distal leg or foot; no exposed bone, unless limited to the distal phalanx	0.6-0.79	70-100	40-59	Local infection involving only the skin and the subcutaneous tissue exclude other causes of an inflammatory response of the skin (*e.g.*, trauma, gout, acute Charcot neuro-osteoarthropathy, fracture, thrombosis, and venous stasis)
2	Deeper ulcer with exposed bone, joint, or tendon generally not involving the heel; shallow heel ulcer without calcaneal involvement, gangrenous changes limited to digits	0.4-0.59	50-70	30-39	Local infection with erythema > 2 cm, or involving structures deeper than skin and subcutaneous tissues (*e.g.*, abscess, osteomyelitis, septic arthritis, and fasciitis), and no systemic inflammatory response signs
3	Extensive, deep ulcers involving forefoot and/or midfoot; deep, full-thickness heel ulcers with or without calcaneal involvement, extensive gangrene involving the forefoot and/or midfoot; full-thickness heel necrosis with calcaneal involvement	≥ 0.39	< 50	< 30	Local infection with signs of SIRS, as manifested by two or more of the following: (1) Temperature > 38 °C or < 36 °C; (2) Heart rate > 90 beats/min; (3) Respiratory rate > 20 breaths/min or $PaCO_2$ < 32 mmHg; and (4) White blood cell count > 12000 or < 4000 cu/mm or 10% immature bands

ABI: Ankle-brachial index; ASP: Ankle systolic pressure; TcPO2: Transcutaneous oxygen pressure; SIRS: Systemic inflammatory response syndrome.

interphalangeal joints show flexion and malpositioning, leading to foot deformity[42,43]. Clinically, motor neuropathy often presents with sensory damage. The combination of motor and sensory neuropathy results in an unequal foot load and insecure gait with pain insensitivity, and the deformed joints and over-pressure-loaded plantar are constantly worn and develop hyperkeratosis over time, promoting the development of ulcers[32,43-45].

Autonomic system dysfunction is thought to be responsible for the pathogenesis of ulceration. Sweating dysfunction caused by autonomic neuropathy causes overheating of the skin through increased deeper blood perfusion, resulting in anhidrotic and fissural skin and a broken dermal barrier

and diminishing the effectiveness of the skin as a barrier against microbial invasion[32,46]. Moreover, the increased glycation of keratin aggravates the ulcers by causing the skin to become thick and squeezing the soft tissue that it covers[47].

PAD

PAD is a clinical term that is classically used to summarize the various diseases that affect the noncardiac and non-intracranial arteries and result in complete or partial occlusion of the peripheral arteries of the upper and/or lower limbs, leading to tissue ischemia and blood supply insufficiency[48,49]. PAD is another equally important contributor to neuropathy in the occurrence of leg ulcers and amputation[50]. The frequency of lower limb amputations in diabetes patients with PAD is higher than that in those without PAD, which may be related to a stronger association with DM in limbs below the knee because the arteries of the lower limbs, especially distal arteries like the dorsalis pedis artery, are mostly involved in DM[51-53]. Among DM patients with PAD characterized by occlusion of the lower limb arteries, one-third will experience intermittent claudication described as pain, cramp, and/or numbness of the affected limb, which occurs when exercising and at rest[52,54]. Long-term intermittent claudication causes progressive dysfunction and disability, and in combination with an impaired vasodilatory response to plantar pressures, it can result in critical limb ischemia, thus leading to foot ischemic ulceration and amputation[55-57].

Foot deformities

Together with neuropathy and trauma, foot deformity was reconfirmed by the Task Force of the Foot Care Interest Group of the American Diabetes Association as a most common triad of causes that interact and ultimately result in ulceration[34,58]. Common structural foot deformities include interphalangeal joint deformity, MTPJ deformity, pes cavus, and pes equinus[59]. The most prevalent and common deformity in DM patients is MTPJ deformity, including hammer-and-claw toes characterized by hyperextension of interphalangeal joints, and hallux valgus characterized by outward tilting of the first MTPJ[58,60].

At present, the specific course of foot deformities in patients with DM is not clear. The widely accepted pathogeny is associated with muscle atrophy, decreased joint mobility, and uneven force on the sole as a result of motor neuropathy[58,59,61]. In DM patients, the musculoskeletal components are destroyed, which is embodied by the atrophy of intrinsic and extrinsic foot muscle and fatty infiltration [62-64]. The atrophy of small muscles like the extensor digitorum brevis and/or interosseous muscles directly affects the stability of joints and the function of the foot by destroying the structure of joints and leading to MTPJ hyperextension and interphalangeal joints hypercurvation[33,65,66]. Moreover, because of incorrect overpressure, the mobility of joints gradually decreases, further aggravating the pressure on the bony prominences, particularly the metatarsal head[67]. Persistent exposure to repetitive and excessive pressure causes deformation of the metatarsal head, and pressures exceeding the threshold may lead to prolonged ischemia, causing the skin below to weaken and break down[68-71]. Meanwhile, blood supply recovery after ischemia caused by pressure changes can lead to reperfusion injury. These ischemia-reperfusion cycles may trigger an excessive inflammatory response, further aggravating the tissue injury, which is considered to be another cause of pressure ulcers[72,73].

Other factors also contribute to ulcer formation by increasing plantar pressure. Hyperkeratosis refers to a thickening of calluses caused by sustained increasing plantar pressure, and is a crucial factor that always precedes ulcer formation[59,74]. Callus thickening has been reported frequently in the plantar area of the metatarsal heads, the heel, and the middle of the big toe[59]. Once formed, it adds gentle but sustained pressure on the underlying soft tissue, and in combination with other pressures, it leads to the formation and rupture of ulcers[58,75]. Another common factor is pathological changes in the tendon, like an increased Achilles tendon size and abnormal tendon structure[76-78]. Thickened fascia and tendon limit joint activity and weaken ankle dorsiflexion, also accelerating the formation of ulcers[62,79].

DFU PREVENTION AND MANAGEMENT

The existing management systems for DFU have gradually expanded on the basis of the three principles established by Treves[80], namely, sharp debridement, offloading, and education. In this section, several commonly used management approaches and their applications are listed, indicating that multidisciplinary DFU care will eventually become the mainstream approach.

Preventive education

Foot care education and self-examination represent the cornerstone and the primary protective factor in DFU prevention[81]. Comprehensive foot care and intensive nursing education together with patient education are reported to be simple, feasible, and strongly effective for DFU prevention[82,83]. For physicians and/or podiatrists, periodic evaluation of arterial perfusion in patients with DM, especially those with peripheral neuropathy and/or foot deformity, which are the main predictive risk factors for

DFU, may help improve the foot condition. For medical institutions, strengthening publicity on preventive measures to improve patients' self-management is important and increasingly urgent[84]. The popularization of self-management should include multiple aspects like foot hygiene instruction, proper footwear use, skin lesion self-examination, and foot sensation self-evaluation. Guiding and encouraging patients to wash feet with water at a moderate temperature, keeping feet clean and dry, and inspecting the condition and checking the color of foot skin can help effectively avoid cracks caused by autonomic neuropathy and usual redness of the skin caused by overpressure[81]. For patients, more than improvements in self-management, regular screening for diabetes complications such as ophthalmic complications are essential and more cost-effective than no screening[82].

Debridement

Debridement can be performed by surgical and non-surgical methods, and both of them are used to remove nonviable or devitalized tissue from the wound bed to accelerate granulation tissue formation and re-epithelialization, which promote wound healing[85]. Experts have considered surgical debridement as the formation of a "new acute wound", since the nonviable tissue has to be debrided down to the bleeding tissue[33]. This mechanical separation is impossible without damaging normal tissues. The surgical removal of superficial necrotic and hyperkeratotic tissue caused by repeated pressure on the foot is essential for wound healing, and it is necessary for deep wounds with bone and soft tissue involvement. Non-surgical debridement includes autolytic debridement with hydrogels, enzymatic debridement, biosurgery, and mechanical debridement with hydrotherapy[86]. Medicinal maggots have shown the ability to remove nonviable tissue selectively and may reduce the risk of secondary superinfection[33], which may lead to a shortened period of wound-healing progression[87].

Glycemic control

The close relationship between blood glucose levels and the progression of diabetes complications has been reported extensively in the literature[88]. Intensive glycemic control in patients with DM has been reported to delay the occurrence of retinopathy, peripheral neuropathy, and nephropathy, all of which are the main risk factors for DFU, and thus show a positive correlation with wound healing. Various studies evaluated and reported the positive correlation of glycemic control and DFU outcomes[39,89,90]. Hemoglobin A1c (HbA1c) is an important clinical predictor of wound healing that shows an increase of 1% when wound healing decreases by 0.028 cm^2. In the Diabetes Control and Complications Trial, intensive glycemic control reduced the incidence of microvascular complications, including DPN, and a 1% decrease in the HbA1c level was accompanied by a 37% reduction in microvascular complications in the United Kingdom Prospective Diabetes Study[91].

Nevertheless, the definition of intensive beneficial glycemic control differs across trials and guidelines. The International Diabetes Federation recommended an HbA1c level lower than 6.5%[92], whereas the American Diabetes Association subdivided and specified the standards for older adults [93], children[94], and pregnant women[95], and recommended an HbA1c goal below 7% for nonpregnant adults[96]. One review of nine randomized controlled trials found that intensive glycemic control based on a target HbA1c level of 6% to 7.5% was associated with a 35% reduction in the risk of amputation in patients with diabetic foot syndrome[97,98].

However, the benefits and adverse effects of intensive glycemic control are still unclear[39]. Acute glycemic control did not show a relationship with the wound outcomes and amputation rate in DFU patients in most studies[98]. The intensity of glycemic control partly determines the incidence of hypoglycemia. In multiple types of studies, a significant adverse consequence of intensive glycemic control was the increasing incidence of hypoglycemia[39,99,100], so intensive glycemic control must also be accompanied by cautious monitoring[36]. However, the lack of clinical evidence and data supporting tight glycemic control should not deter efforts to achieve the target of optimal glycemic control, since it has been suggested to be the only significant tool to prevent complications in patients with both type 1 and type 2 diabetes[101].

Since uncontrolled hyperglycemia is one of the reasons why the readmission rate of DFU patients is as high as 30%, which is much higher than that of other patients, intensive glycemic control will help prevent such readmissions[102,103]. Besides, intensive glycemic control will help form a "glycemic memory" or "legacy effect", which implies that the benefits of earlier interventions are still evident while following the disease course[104].

Exercise

The effect of exercise on DFU is probably mediated by its effects on the risk factors. Exercise is reported to play a role in preventing or counteracting PAD in patients with type 2 DM[55], since regular physical activity may improve the claudication distance in PAD[50]. Moreover, exercise can disrupt the progression of DPN. Different types of exercise have significant effects on HbA1c reduction, and combined exercise is more effective in comparison with aerobic and resistance exercise[55]. In future studies, the exact relationship between exercise and DFU therapy should be determined to allow better integration of exercise into the treatment.

Offloading

Evidence-based guidelines have reported that reducing high foot pressure (*i.e.*, offloading) is the main objective and a significant prerequisite for promoting the healing effect and preventing ulcer[105,106]; this process involves offloading the affected area of the foot by redistributing extra pressure to other regions[107]. The majority of offloading device interventions are available for DFU and are divided into four categories: Casting, bracing, footwear, and walking aids[108]. In this section, four representative offloading devices will be introduced.

Total contact cast: The total contact cast (TCC) is often considered the gold standard device[86], and has been recommended by the guideline as the first-choice treatment option[106,109]. It protects the foot from further trauma and deformity, helps redistribution of excessive pressure[110], promotes tissue repair, and provides a protective load through below-knee-immobilization[111]. In comparison with some other approaches like removable cast walkers (RCWs) and therapeutic footwear, TCCs are reported to offer a better healing rate[108,112,113].

However, despite the substantial effectiveness of TCCs and their attractive characteristics for offloading interventions, their actual utilization rate is far from ideal. In a nationwide survey in the United States, only 1.7% of 858 centers considered a TCC as a the primary offloading method in DFU treatment[114]. Moreover, 45.5% of centers nationwide reported never using the TCC as an offloading modality, and 58.1% of centers did not consider TCCs as the first choice in noninfected plantar DFU treatment[114].

The low utilization rate can be attributed to a complex interplay of multiple factors. For patients with DFU, TCC is not easy to disassemble, which ensures their fixation and stability but hampers daily wound care if new pressure ulcers occur, hinders mobility, and results in inconvenient application because of the need for skilled technicians[107]. In addition, prolonged casting can cause stiffness of the muscles and atrophy of the joints[111], potentially leading to low patient acceptance. For medical institutions and physicians, the lack of awareness or familiarity with guidelines, the unpredictable efficacy, the inertia associated with previous practices, and the lack of skilled technicians may lead to a low level of TCC use.

RCW: A RCW is a removable knee-high offloading device. It offers multiple advantages, including easy removability, convenient wound assessments and care, and comfortable movement in daily life[115]. In comparison with TCCs, the most significant advantage of RCW is the reduction in time, energy, and experience needed for proper application[116], which makes it more suitable for frequent examination and nursing in cases of new ulcer occurrence and after an operation.

RCWs provide an equal level of plantar pressure and wound healing as TCCs and have emerged as a potential alternative to TCCs[117,118]. However, the convenience of removable RCWs may be obtained at the expense of healing ability. In *in vivo* studies, RCWs showed significantly lower healing ability in comparison with non-removable knee-high offloading devices like TCCs[117]. This significant difference in healing ability may also be caused by patients' different compliance levels while wearing the device, since patients' adherence to using the devices can promote healing. Under these circumstances, while the convenient application and removal is the greatest advantage of RCW, it also reduces the patients' compliance since the TCC cannot be removed by the patients themselves, while the RCW can[114]. Patients may be unwilling to wear the device at home, so the noncompliance in using the RCW directly affects the healing process[119].

Therapeutic footwear: Proper footwear has long been considered to play an important role in DFU care [120]. Therapeutic footwear is considered an effective approach for ulcer healing and has been used as a DFU-prevention strategy for decades[86,120]. It has been generally divided into several parts like a shoe, insole, and felted foam[108,111]. Typical diabetic prescription shoes usually have a deeper, looser, rocker outsole and toe box with soft support padding and can provide better accommodation for foot deformities[121,111]. Treatment with therapeutic shoes has been reported to yield reduced relapse in comparison with non-prescription shoes[122]. Forefoot offloading shoes (FOS) are representative prescription shoes specifically designed to offload the forefoot and have been proven to be efficacious in offloading and healing diabetic plantar forefoot ulcers. FOS mainly consist of a rocker bottom outsole and a negative-heel configuration that limits active dorsiflexion of the toes and shifts weight-bearing proximally, redistributing the load of the forefoot[107]. In comparison with standard prescription shoes, FOS reduce forefoot peak pressure ranging from 15% to 20%[123] and are recommended after surgery to offload the forefoot in case of injuries and ulcers. However, the negative-heel rocker-outsole design of FOS may compromise gait symmetry and stability, potentially decreasing wearing comfort and clinical acceptance[124,125].

Insoles have been reported to show good results in reducing shear or side-to-side stresses on the foot plantar surface, which is another key factor in DFU prevention[126]. Shear-reducing insoles are similar to dynamic foot orthosis (DFO) insoles. These insoles are composed of a free-floating distal segment and anterior segment that slide over each other[127]. This special structure is designed to reduce the shear stress on both the foot and insole. Meanwhile, a reduction in the midfoot temperature increase was observed after using DFO insoles, and since a regional foot temperature increase is associated with ulcer

formation, these findings demonstrated the protective effects of DFO insoles in DFU formation.

As one of the most commonly used accommodative dressings, the combination of felted foam with other therapeutic footwear is considered a promising approach to promote ulcer healing. Zimny *et al* [128] evaluated the effect of felted foam on wound healing in comparison with classical pressure-reducing devices and confirmed its promoting effect. Nubé *et al*[129] found that felt deflective padding applied to both skin and shoes provided similar wound-healing promoting effects for small, primarily neuropathic ulcers. Felts of different materials also influenced the healing of wounds. Pabón-Carrasco *et al*[130] reported that a combination of latex-wool felts showed great pressure-reducing ability, potentially combining wool's timely pressure capacity and latex's durability and structural stability. In comparison with wool, polyurethane, and latex, latex-wool felts offer the comprehensive advantages of hybrid materials and can serve as a great substitute for single material like wool.

In conclusion, published studies recommend the use of unremovable devices like TCCs for DFU offloading. When unremovable devices are unsuitable because of social, economic, and/or patient psychological factors and acceptance, removable devices like RCW can be used to address treatment adherence since they have the same level of therapeutic effect as unremovable devices[131]. For physicians, when choosing therapeutic footwear to assist therapy, more consideration and analysis should be paid to the specific offloading location of the foot and adherence to using offloading devices clinically[132].

Surgery

Deformities that develop into DFU commonly include hammertoes, prominent metatarsal heads, and hallux limitus[133]. A fixed-location high plantar pressure caused by structural deformities can be a predisposing risk factor for DFU recurrence if it is not adequately offloaded by the abovementioned conservative non-surgical offloading approaches. In such cases, foot surgery to ameliorate the overpressure through structural reorganization or removal of the underlying bony prominences is essential[134]. For patients showing chronic deformities and ulcers, foot surgery interventions are an important component in the management of foot ulcers, and can help them get rid of wearing cumbersome braces or footwear[133].

The offloading surgeries identified in IWDGF predominantly include tendon procedures such as toe flexor tenotomy and Achilles tendon release, but other types of surgeries can also be performed to relieve plantar pressure. Foot surgery has been classified into different types on the basis of the clinical conditions. Armstrong *et al*[135] revised a foot surgery classification system based on the presence of open wounds and acuity, and the conceptual framework of the surgery definitions in their study was based on the risk of high-level amputation. This system classifies foot surgery into four classes: Class I refers to elective surgeries aimed at reconstructing a deformed foot for patients without neuropathy, class II refers to prophylactic surgery aimed at reducing the risk of recurrent ulceration for patients with neuropathy but no open wound, class III refers to curative surgery aimed at offloading the overpressure caused by bony prominences and draining the underlying abscesses for patients with open wounds, and class IV refers to emergent surgery aimed at controlling infections caused by wet gangrene, necrotizing fasciitis, *etc.* for patients with severe infections[135].

Ahluwalia *et al*[136] systematically analyzed and summarized the five discrete types of offloading surgeries usually employed in cases of recalcitrant ulcers: (1) Lesser toe tenotomies, which aim to release the tight flexor tendon and decompress a flexible hammer toe for patients with recalcitrant ulcers on the tip or the knuckle of a deformed toe; (2) Achilles tendon release and metatarsal offloading, which aim to promote ulcer healing by releasing the Achilles tendon, metatarsal head resection(s), or joint arthroplasty; (3) Hallux procedures, which aim to redistribute the forefoot pressure by resetting the first metatarsal-phalangeal or partly amputating the hallux; (4) Surgical mastectomy, which aims to offload the overload area by directly removing the bony prominences in patients with a stable, inactive Charcot deformity; and (5) Complex surgical foot reconstruction, which aims to build a stable foot structure that can help patients walk normally without pressure areas[136].

Regular postoperative care is another extremely important aspect influencing ulcer recurrence and prevention of amputation. The reported complications after exostectomy include wound non-healing, wound dehiscence, and skin and soft tissue infection, all of which will increase ulcer recurrence and amputation rates[137]. In this regard, 70% of DFU patients have been reported to show a second ulcer recurrence after discharge, directly leading to amputation[32]. Therefore, meticulous wound care, adequate nutrition, and appropriate post-care management are essential for patients presenting with DFU, especially those who have undergone foot surgery.

Infection control

The bacterial toxins in wounds can cause infection, leading to collagen degradation, stress, and malnutrition and thereby preventing wound healing, which is a known predictor of poor prognosis and amputation[138]. Thus, correct identification and appropriate control of infections is essential to improve the prognosis in patients with DFU[86]. Diabetic foot infection (DFI) is particularly difficult to manage because the absence of exact markers to measure the level of microbiological activity for a typically colonized wound forces diagnosis based on clinical judgment[139], which often depends on the characteristics of inflammation such as per ulcer redness or induration and increased purulent

drainage[140].

In the early stage, DFU usually shows monomicrobial infections, while polymicrobial infections are observed in the middle-to-late stages[141]. Polymicrobial infections and their interactions in the DFU can delay or even stop wound healing[142]. Current clinical guidelines recommend systemic antimicrobial therapy for patients with DFI[85,139], and the formulation of a specific medication regimen is important in this regard. In the guideline developed by the Infectious Diseases Society of America (IDSA), the antibiotic regimen usually depends on the degree of infection, *e.g.*, using antibiotics targeting aerobic Gram-positive cocci for patients with mild-to-moderate infections and broad-spectrum empirical antibiotic therapy for patients with severe infections[85]. The appropriate use of antibiotics plays an important role in the prognosis of DFU, and improper or excessive antibiotic usage may cause several side effects like antibiotic resistance. IDSA advised that to avoid the adverse consequences of antibiotic overuse, narrow-spectrum antibiotics should be used for clinical treatment over the shortest term possible and discontinued immediately after the symptoms have been resolved[85].

Assessment of life quality

To avoid problems with treatment acceptance and compliance, the treatment of DFU should not only be limited to objective medical evaluation but should also include consideration of the patients' subjective feelings[143]. Assessment of the health-related QoL of patients is becoming steadily more important, especially in the treatment and evaluation of chronic diseases with a high prevalence, and should be an integral part of clinical evaluations of the prognosis of diabetes and its complications. All aspects, including physical health, pain, difficulty with usual activities, social function, role emotional, *etc.*, should be considered when evaluating the prognosis of a patient[144]. In DM patients, reductions in QoL will worsen in the presence of complications[144] such as DFU since these complications can limit physical functions such as mobility and cause pain, thereby increasing the psychological burdens caused by limitations in social relationships and fear of amputation, reducing patients' compliance with treatment, and eventually decreasing the survival rate[145-147].

Different treatment measures have shown different effects on patients' QoL. The chronicity of DM causes patients to show a higher possibility of developing psychological disorders, which is more obvious in patients who have undergone a major amputation[148]. Moreover, studies have reported significantly worse stress readaptation and deterioration of glycemic control after amputation[149], which reduces patients' QoL and weakens their socio-economic status[144]. Physical activity and exercise were confirmed to effectively improve DFU-related psychological pressure. One study reported improvements in glucose control, balance, neuropathic symptoms, and QoL of patients with DPN after Tai Chi exercises[150]. In combination with other related studies, these findings showed that patients in exercise programs have better QoL in terms of physical fitness, social ability, and emotional pressure [151].

Since offloading devices are one of the commonly used treatment modalities for DFUs, the differential influence of different types of devices on QoL should be considered clinically[152]. Although offloading devices redistribute plantar pressure and improve foot health, the accompanying adverse effects on gait and mobility should not be underestimated. Therapeutic footwear, especially when used on only one side, will cause the patient to limp while walking, causing deterioration of gait speed and symmetry, stride length, and the gait cycle time of patients with DFU. To reduce the related gait disorders and improve the patients' QoL, the use of bilateral therapeutic shoes instead of unilateral shoes can be a better option[153]. Casts show a good therapeutic effect because of their sealing ability and protective effects on wounds, which may be the reason for the higher cure rate of TCC in comparison with standard treatments[154,155]. However, the low patient acceptance of TCC is because of the limitations that it imposes on daily activities, as well as the difficulties in wound care and observation[113]. In contrast, the easy disassembly of RCW makes wound care and daily activities much more convenient, making it more acceptable for patients with DFU[114].

The QoL associated with a treatment method determines the extent to which it will be accepted and used by patients and should be one of the basic considerations when choosing therapeutic options. Currently, differences in QoL associated with different therapies have not received much attention, judging from the limited research on the relevant aspects and guidelines[156]. More studies should focus on QoL assessments to help formulate more reasonable clinical treatment plans.

CONCLUSION

DFU is a common and growing problem worldwide. The treatment approach for DFU depends on a combination of various factors that have been listed and discussed in this article. The following aspects should be considered to prevent ulcer progression and promote ulcer healing: (1) Choosing a proper classification to summarize the clinical details for further management and for auditing clinical outcomes; (2) Investigating risk factors that may predict the occurrence and promote the progression of ulcers; and (3) Employing validated interdisciplinary DFU management and care pathways, and emphasizing the cultivation of patient compliance. The findings highlight the need for the development

and application of more relevant prevention and treatment measures in the clinical management of DFU.

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REVIEW

Mesenchymal stem cell-derived exosomes: The dawn of diabetic wound healing

Jing Wu, Li-Hong Chen, Shi-Yi Sun, Yan Li, Xing-Wu Ran

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Abstract

Chronic wound healing has long been an unmet medical need in the field of wound repair, with diabetes being one of the major etiologies. Diabetic chronic wounds (DCWs), especially diabetic foot ulcers, are one of the most threatening chronic complications of diabetes. Although the treatment strategies, drugs, and dressings for DCWs have made great progress, they remain ineffective in some patients with refractory wounds. Stem cell-based therapies have achieved specific efficacy in various fields, with mesenchymal stem cells (MSCs) being the most widely used. Although MSCs have achieved good feedback in preclinical studies and clinical trials in the treatment of cutaneous wounds or other situations, the potential safety concerns associated with allogeneic/autologous stem cells and unknown long-term health effects need further attention and supervision. Recent studies have reported that stem cells mainly exert their trauma repair effects through paracrine secretion, and exosomes play an important role in intercellular communication as their main bioactive component. MSC-derived exosomes (MSC-Exos) inherit the powerful inflammation and immune modulation, angiogenesis, cell proliferation and migration promotion, oxidative stress alleviation, collagen remodeling imbalances regulation of their parental cells, and can avoid the potential risks of direct stem cell transplantation to a large extent, thus demonstrating promising performance as novel "cell-free" therapies in chronic wounds. This review aimed to elucidate the potential mechanism and update the progress of MSC-Exos in DCW healing, thereby providing new therapeutic directions for DCWs that are difficult to be cured using conventional therapy.

Key Words: Diabetic wounds; Wound and injuries; Mesenchymal stem cells; Exosomes; Pre-conditioning; Preclinical translation

Core Tip: Diabetic chronic wounds (DCWs) are one of the most serious chronic complications of diabetes, and the efficacy of stem cell therapies for refractory chronic wounds has been studied previously. Stem cell-derived exosomes are one of the important active components of stem cell paracrine secretion, which inherit the wound repair capacity of parental cells as parts of novel cell-free therapies in addition to cell-bases ones. Herein we discuss the mechanism and latest progress of mesenchymal stem cell-derived exosomes in promoting DCW healing.

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INTRODUCTION

Wound healing after skin tissue injury relies on a dynamic chain of physiological reactions including hemostasis, inflammation, cell proliferation, and tissue remodeling[1]. Any step out of balance, such as excessive inflammation, impaired fibroblast migration and proliferation, abnormal collagen formation and deposition, and hindered re-epithelialization, ultimately leads to delayed wound healing and formation of chronic wounds. Chronic wounds are those that have failed to proceed through an orderly and timely reparative process to produce anatomical and functional integrity of the injured site[2]. They refer to wounds caused by multiple factors that have not healed or have not demonstrated a tendency to heal after a certain period clinically, with a chronic duration ranging from 4 to 12 wk[3,4]. Various pathological states result in chronic wound development, including diabetes, pressure injuries, infections, and arterial/venous insufficiency of which reports are similar in China and developed Western countries[4-6], which have the most complicated pathogenesis and therapeutic strategies being diabetic chronic wounds (DCWs).

Diabetes mellitus (DM) is a metabolic disease characterized by elevated blood glucose levels, of which DCWs are among the most threatening complications. The combination of a high-glucose environment and several biological factors, including ischemia and hypoxia, abnormal inflammatory response, excessive oxidative stress, and peripheral neuropathy, contributes to wound formation[7-9]. Such wounds have problems of protracted healing, long treatment time, difficulties in management, high cost, repeated attacks, and high disability/mortality rates, resulting in heavy physical, psychological and economic burdens[10,11]. The intervention of DCWs cannot be underestimated based on what is mentioned above. Hence, solving persistent inflammation, impaired cell proliferation and migration, decreased angiogenesis, and remodeling of the extracellular matrix (ECM) is important. Innovative wound repair methods, such as local negative pressure, growth factors, and autologous platelet-rich gels, have remarkable effects on healing DCWs[12-15]. However, more specific treatment options are required for refractory and contraindicated wounds.

With the rapid development of tissue engineering, cell therapies have gradually become widely used in various disciplines. Stem cells can be used in regenerative medicine and play an indispensable role in wound repair[16], of which mesenchymal stem cells (MSCs) are the most commonly used. MSCs have self-renewal abilities and multi-directional differentiation potential, participating in damage repair through intercellular communication and bioactive factor secretion, finally achieving the effect of promoting wound healing[17]. Clinical trials of MSCs for treating various types of cutaneous wounds are currently in full swing, and their efficacy and safety in promoting wound regeneration have been initially demonstrated. As clinical trials continue to progress, further attention and supervision need to be paid to their potential safety issues of proliferative lesion formation, abnormal organ reaction and unknown long-term health effects after transplantation[18-20].

Studies have revealed stem cells promote repair and regeneration mainly through paracrine signaling, whereas exosomes are one of their important paracrine active components[21]. MSC-derived exosomes (MSC-Exos) carry genetic information, functional RNAs, and proteins from parental cells, demonstrating wound healing effects *via* intercellular communication after these biologically active substances are acquired by recipient cells[22-24]. Thus, MSC-Exos have broad application prospects in diabetic wound repair[25]; however, they have not yet been carried out in clinical practice. The important role of MSC-Exos in all stages of diabetic wound healing and the preclinical application are highlighted in this review, to pave the way for their use as an effective tool in the management of these harmful diabetic complications.

DCWS: HEALING DISORDERS CAUSED BY VARIOUS MECHANISMS

DM is a metabolic disease characterized by elevated blood glucose levels, which poses a serious threat to human health. The continuous progression of hyperglycemic toxicity without effective control will affect macrovascular, microvascular, and peripheral nerves throughout the body and involve various organs such as the brain, eyes, heart, kidney, and skin, resulting in various diabetic chronic complications[26]. DCWs are one of the most common and threatening chronic complications, often accompanied by infection or deep-tissue destruction[27]. Protracted wounds are the most common cause of non-traumatic amputations. Diabetic foot ulcers (DFUs) are characterized by wounds on the feet, which are the most typical, and patients with DFUs have a 2.5 times higher risk of 5-year mortality than those with none[28]. The overall mortality of DFUs within 5 years is nearly 50%[29], and approximately 20% of moderate-to-severe DFUs will lead to amputation; the 5-year mortality rate after amputation exceeds 70%[30].

Impaired wound healing processes caused by hyperglycemia-induced disturbances in wound-linked cellular behaviors contribute to diabetic wound healing difficulties[7,31]. Hyperglycemia, oxidative stress, and insulin resistance affect the function of vascular smooth muscle cells, endothelial cells, and platelets, which in turn may lead to abnormal coagulation processes and affect platelets of triggering for subsequent inflammatory and proliferative phases[32]. The hyperglycemic microenvironment can lead to dysfunction of immune and inflammatory cells and dysregulation of inflammatory factors. Perpetuated inflammatory states induced by increased mast cell degranulation[33], excessive extracellular traps produced by neutrophils[34], dysregulated and persistent M1 (pro-inflammatory) macrophage polarization[35], pro-inflammatory factors (IL-1β, TNF-α, and IL-6) overexpression, and anti-inflammatory factors (IL-10 and TGF-β) deficiency finally hinder wound healing[7]. The proliferative phase of diabetic wound healing is characterized by disturbed physiological functions of keratinocytes[36], fibroblasts[37], and endothelial cells[38], then the impaired re-epithelialization, granulation tissue formation, matrix deposition, and angiogenesis affect wound healing. Various factors also affect the function and activity of these cells during this phase, including decreased chemokines with pro-angiogenesis produced by macrophages, hemoglobin glycation, vascular stenosis, increased oxygen consumption affecting oxygen-dependent cellular behaviors, and impaired nerve fiber regeneration[7,31,39,40]. Remodeling of the ECM spans the entire injury response, and fibroblasts are the major cell type responsible for this phase[31]. Sequential changes in the ECM require a balance between collagen degradation and synthesis, achieved through temporal regulation of the dynamic changes in the ratio of matrix metalloproteinases (MMPs) to tissue inhibitors of metalloproteinases (TIMPs)[41,42]. Such changes in DCWs are unbalanced and lead to difficult wound healing and excessive scarring[41,43]. However, no clear demarcation exists between the various stages of wound healing, and functionally impaired cells can interact, eventually leading to poor diabetic wound healing, progressing to local infection, gangrene, and even amputation. Therefore, the most important aspect of effectively treating DCWs is to identify an appropriate approach that can comprehensively improve abnormalities in all phases of wound healing.

CURRENT STRATEGIES AND PROMISING DIRECTIONS FOR DCWS REPAIR

Traditional strategies for DCWs management include glycemic control, conventional dressings (*e.g.*, hydrocolloids, alginates, and silver ions, *etc.*), thorough debridement (*e.g.*, surgical, mechanical, ultrasonic waterjet, collagenase, and maggot, *etc.*), wound off-loading, autologous skin and skin substitute grafting, infection control, and revascularization, *etc.* These strategies are used to create the wound bed microenvironment suitable for repair through moisture balance maintenance, necrotic or inactivated tissues removal, systemic and local infections control, and local blood flow improvement[13,44-46]. Negative pressure wound therapy can also be used to achieve its role in improving wound exudate drainage, enhancing local perfusion, removing bacterial products, promoting granulation tissue growth, and facilitating wound healing[47]. However, these conventional treatments are often ineffective in many patients because of impaired cell function around the wound sites caused by underlying microenvironmental alterations[48]. Several innovative wound adjuvant therapies, including exogenous supplementation of growth factors[49], platelet-rich plasma[50], autologous platelet-rich gels[15,51], and hyperbaric oxygen therapy[52] have been developed to promote the activity and function of damaged cells and offer the possibility of treating unselected refractory wounds. However, an updated systematic review has revealed that some measures had positive effects on accelerating wound healing, while others had limited impacts on diabetic ulcer healing[53]. However, the overall efficacy of various treatment modalities for DCWs remains unsatisfactory, and effective therapeutic strategies need to be continued.

STEM CELL-BASED THERAPIES BECOME HOT TOPICS, COMING EXOSOMES INTO BEING

Stem cells have the potential for self-renewal and multidirectional differentiation with great research and application value in life sciences, clinical trials and disease research. Stem cell-based therapies are now approved by several countries, and have been widely used in various disciplines. MSCs are currently the main experimental cell sources and have shown their excellent therapeutic potential and value in clinical trials in the field of regenerative medicine[16,54].

MSCs provide assistance in all phases of wound healing by exerting their functions of regulating skin homeostasis and wound healing through migration into the skin damage site and interaction with skin cells and can influence the function of these cells by paracrine secretion of bioactive factors and differentiation into them[55,56]. As MSCs have exhibited wound healing in many preclinical studies as powerful tools for regulating inflammation, promoting cell proliferation and migration, angiogenesis, and collagen synthesis[57-60], the application of MSCs for DCWs contributes to progress toward clinical trials. Twenty-five clinical trials of MSCs for diabetic ulcers have been conducted or are recruiting subjects, which are recorded in the ClinicalTrials.gov database (clinicaltrials.gov).

Previous clinical studies have demonstrated that MSC transplantation in patients with DFUs is safe and feasible with the properties of improving microcirculation, wound healing, ulcer recurrence, and amputation[61-63]. However, stem cell therapies are still in their early clinical stage, further attention and supervision are required of declined performance during production and application as cellular senescence and loss of multipotency during *ex vivo* expansion and from variable donors[64,65], decreased survival rate caused by advanced glycosylation end products[66], potential safety issues as proliferative lesion formation and abnormal organ reaction[20], and unknown long-term health effects after transplantation. Basic and clinical researches related to allogeneic/autologous stem cells are subject to the International Society for Stem Cell Research Guidelines for Clinical Translation of Stem Cells and national ethical guidelines and related guidelines/regulations[20,67].

MSCs exert their repair and regenerative effects mainly through paracrine signaling, and exosomes are one of the important active components[21] that provide a more stable entity that minimizes the potential safety concerns for cell transplantation. MSC-Exos play an important role in intercellular communication by carrying various important functional substances of parental cells, being used of promoting wound healing[68,69]. Compared to direct cell transplantation, MSC-Exos avoid the immune rejection because of low immunogenicity; allow to cross various biological barriers and avoid the risk of embolism from intravenous injection based on their smaller sizes[70]; the dose and fraction can be adjusted artificially and genetic modifications are easier and safer[71]; avoid the problem of malignant transformation; and allow to repair diabetic complications through multiple actions[72]. They can also be used as ideal carriers for carrying and delivering therapeutic drugs, genes, enzymes, or RNAs[73], and their efficiency and targeted transport capacity can be tuned through pretreatment or engineering transformation[74], demonstrating their promising applications in the field of repair and regeneration.

STEM CELL-DERIVED EXOSOMES: NOVEL CELL-FREE STRATEGIES

Exosomes biology

The concept of "exosomes" was first proposed in 1981 by Trams *et al*[75], using to collectively refer to extracellular vesicles (EVs) that originated from the exudation of various cell line cultures. The currently defined exosomes were first discovered in sheep reticulocytes and considered cellular waste[76-78]. Of note, "EVs" is the preferred term by the International Society for Extracellular Vesicles (ISEV) to describe all nanoparticles with lipid bilayer structures released by cells[79].

Exosomes, the biological nanoscale spherical lipid bilayer vesicles[80], can be secreted by almost all cell types and are widely present in cell culture supernatants and many body fluids[81]. Their diameters range from 10 to 200 nm. In addition to exosomes, EVs also include microvesicles that are also called ectosomes with a diameter of 100-1000 nm, and apoptotic bodies larger than 1000 nm according to different sizes and biogenesis[82,83]. The types and functions of the bioactive substances carried by exosomes differ according to their cellular origins and adjacent cellular components[84]. The major substances include genetic information, RNA species (mRNA, tRNA, rRNA, miRNA, lncRNA, circRNA, *etc*.), proteins, lipids, cytokines, and growth factors[85,86]. Exosomal proteins include intrinsic components involved in exosome biogenesis, such as fusion-related proteins (GTPases, annexins, flotillin, and Rab proteins), heat shock proteins (HSP70 and HSP90), tetraspanins (CD63, CD81, CD82, and CD9), ESCRT complex, and specific functional proteins originating from parental cells[87]. Apart from serving as a medium for cellular communication, some proteins are also involved in the membrane composition and biosynthesis as identified biomarker proteins and can provide stability and permeability in concert with phospholipid bilayers.

Exosomes originate from endosomes during generation, circulation, degradation, and liberation[88]. Extracellular substances fuse with early sorting endosomes through plasma membrane invagination and

endocytosis, and begin to accumulate bioactive substances. Eventually, they mature into late sorting endosomes, which invaginate to form intraluminal vesicles that can then generate multivesicular bodies (MVBs)[68,88]. MVBs can be absorbed by lysosomes comprising a degradative pathway, or they can undergo a specific exocytotic process whereby they fuse with the plasma membrane to release exosomes into the extracellular space[89]. After release, they act as mediators of intercellular and intra-organ communication to transfer the contained bioactive substances to recipient cells through direct fusion, endocytosis, and receptor-ligand binding to affect their functions[90,91], participating in the body's physiological and pathological state adjustment[92].

Isolation and characterization of exosomes

The extraction of exosomes is primarily based on their physicochemical properties. This process is difficult because of the heterogeneity of exosomes derived from different cell origins, the possible existence of subpopulations of exosomes with different functions and phenotypes even when extracted from a single cell line, and multiple EV subtypes with similar biophysical properties[93]. Therefore, different isolation methods should be targeted for different purposes[87]. Differential ultracentrifugation is the most widely used separation technique and is also known as the gold standard for isolation, while the main principle is to harvest the desired components based on size and density differences[94]. Polymer precipitation uses polyethylene glycol to harvest exosomes under centrifugal conditions by reducing their solubility[95]. Size-exclusion chromatography[96] and ultrafiltration[97] are both based on size differences between exosomes and other components, although they may adulterate other particles of similar size. Immunoaffinity capture is based on the specific binding of antibodies and ligands to isolate exosomes from a heterogeneous mixture[98]. Current isolation and purification techniques have varying effects and many problems such as low purity and recovery, structural damage, and time and cost consumption, making achieving efficient enrichment difficult, which has become a bottleneck of the translational applications of exosomes[87]. Hence, continuously exploring new isolation and purification techniques or combining multiple techniques is necessary to improve the isolation efficiency and thus obtain ideal exosomes.

Exosomes are mainly characterized by external characteristics (morphology and size detection) and the identification of surface markers[87]. As mentioned above, some protein components of exosomes serve as surface protein markers for identification. The ISEV has proposed the need to identify two types of proteins as follows: one is the biomarker proteins shared by exosomes to determine whether the extracted components are exosomes, and the other is cell-type-specific exosomal proteins that need to be identified to determine cellular origin[79]. Therefore, exosomes can be characterized by detecting their morphology using transmission electron microscopy, their size and concentration by dynamic light scattering, and nanoparticle tracking analysis technology, and their marker proteins by western blot, enzyme-linked immunoassay, and flow cytometry[87].

Biological functions of MSC-Exos

Stem cells have self-renewal abilities and multi-directional differentiation potential, while MSCs are one of the most frequently used and promising adult stem cells that can be derived from most adult tissues such as the bone marrow, adipose tissue, and umbilical cord[99,100]. Bone marrow-derived MSC-Exos (BMSC-Exos) are biologically stable, have low immunogenicity, and exhibit good proliferation and viability after transplantation. They are most commonly used in clinical trials and can play a prominent role in various disorders, especially bone-related diseases[101]. Umbilical cord-derived MSC-Exos (UCMSC-Exos) can be isolated non-invasively, with low immunogenicity and strong self-renewal and proliferation ability, although it has limitations in maintaining bioactive and clinical therapeutic transport[102]. Adipose-derived MSC-Exos (AMSC-Exos) have relatively abundant sources that can be easily obtained by painless minimally invasive surgery; they are also pluripotent, plastic, easy to store, and stable in blood or body fluids[103]. Exosomes of different origins share most of their bioactive factors and are generally similar in their biological functions; however, their specific biological properties depend on the molecules that are specifically expressed[104].

MSC-Exos are involved in intercellular communication through the transfer of proteins, RNA, DNA, and bioactive lipids that can be delivered to target cells to regulate their activities and functions[68]. They are generally involved in the regulation of cell survival and differentiation, the immune system, and inflammation modulation, and are also capable of promoting angiogenesis and tissue remodeling [73]. Considering these multiple biological functions, several studies have also reported that the MSC-Exos play a therapeutic role in autoimmune diseases[105], ischemic injuries[106], and metabolic diseases [107], and are also related to dynamically modulating tumor biological functions[108], promoting repair and regeneration of damaged osteochondral, neural, and tendon tissues, and facilitating wound healing [109-112]. Current studies also discovered that they can improve COVID-19-related cytokine storms and the deterioration of lung function due to severe pneumonia[113].

MSC-EXOS FOR REPAIRING DIABETIC WOUNDS

MSC-Exos play an important role in each phase of wound healing[81]. They can regulate diverse cell types related to wound repair by enhancing or suppressing certain bioactivities, achieving hemostasis, inflammatory regulation, cell migration to the wound site, cell proliferation, and differentiation to form granulation tissue, angiogenesis, and ECM reorganization[69]. They can also be expected to be therapeutic agents for different types of diabetes by alleviating autoimmune damages[114], attenuating insulin resistance, and improving β-cell exhaustion[115]. Additionally, they can be used to prevent and treat DM-related complications. Based on these potentials, MSC-Exos may be of considerable importance in DCW treatment.

Hemostasis

Tissue factor (TF) is an initiator of coagulation activation and was identified in the plasma membrane of exosomes[116]. TF can transfer to the platelets and initiate the extrinsic coagulation cascade, leading to the conversion of prothrombin to thrombin and fibrin clot formation[117]. Induced coagulation and stimulated thrombogenicity were observed using EVs carrying TF from the pericardial blood of patients who received cardiac surgery[116]. Rat BMSC-Exos were applied to the bleeding site in the hemorrhage liver model, which exhibited an inhibited amount of bleeding and shortened bleeding time, demonstrating their excellent hemostatic properties. However, no studies related to exosomes' promotion of coagulation in cutaneous wound healing have been conducted. Further studies are needed to demonstrate the potential role of exosomes in the hemostasis phase of wound healing.

Inflammation

Excessive inflammation is a major cause of persistent diabetic wounds. Abnormal macrophage polarization and cytokine overexpression lead to an uncontrolled and persistent inflammatory state and can cause secondary tissue damage[7]. MSCs-Exos can inhibit the differentiation, activation, and proliferation of T cells as well as reduce IFN-γ release[118]. They can reduce the concentration of the inflammatory cytokines, TNF-α, iNOS, IL-1β, and IL-6[119] and upregulate the expression of the anti-inflammatory cytokine IL-10[120,121]. MSCs-Exos can also induce M2 polarization of macrophages to promote wound healing by delivering exosome-derived miR-223 to target regulating the expression of pknox1 protein[122].

Such abilities can also be observed in diabetic wounds. Topical application of native AMSC-Exos to diabetic mice dorsal full-thickness skin wounds also downregulated inflammatory cytokines (IL-6, TNF-α, CD14, CD19, and CD68) expression and promoted wound healing[123]. Similar alleviated inflammatory effects achieved by regulating inflammatory factors could also be observed in the combination of intraperitoneal Nrf2 pharmaceutical activator and BMSC-Exos subcutaneous injection, demonstrating decreased inflammatory cytokines TNF-α and IL-1β and increased anti-inflammatory cytokines IL-4 and IL-10[124]. Intradermal injection of MSC-Exos derived from human menstrual blood could induce macrophage polarization from the M1 to M2 phenotype, while this capacity is better than that of menstrual blood-derived MSCs[125]. Significantly lower M1 polarized macrophages and higher M2 polarized macrophages were also observed in the diabetic mouse air pouch model and diabetic rat full-thickness skin wound model using BMSC-Exos, while melatonin-stimulated BMSC-Exos (MT-Exos) had stronger effects[121]. Immunomodulatory capacity was enhanced after preconditioning. Moreover, MT-Exos could improve wound healing by activating the PTEN/PI3K/AKT signaling pathway to promote macrophage M2 polarization, angiogenesis, and collagen synthesis; promote the resolution of persistent inflammation; and drive the transition from inflammation to proliferation[121]. HUCMSC-Exos pretreated with lipopolysaccharides have better regulatory properties for macrophage polarization and resolution of chronic inflammation by transferring miR-let7b, while the TLR4/NF-κB/STAT3/AKT pathway is important in regulating this mechanism to promote wound healing[126]. The use of engineered TNF-α/hypoxia-pretreated HUVMSC-Exos in infected DCWs also decreased proinflammatory cytokines (TNF-α, IL-1β, and IL-6), induced M2 macrophage polarization, reduced bacterial burden, and bacterial colonization at the wound sites. Reduced levels of oxidative biomarkers and increased levels of antioxidant mediators also demonstrated the ability of oxidative stress suppression [127]. The combination of BMSC-Exos and carboxyethyl chitosan-dialdehyde carboxymethyl cellulose hydrogel revealed skewed macrophage functional polarity from M1 toward an anti-inflammatory M2 phenotype, as well as enhanced antibacterial effects by significantly inhibiting bacterial growth[128].

Proliferation

Fibroblasts, keratinocytes, and endothelial cells participate in the proliferative phase. Unlike the dual regulatory effects on the tumor, MSC-Exos directly affect the proliferative phase of wound healing by stimulating the proliferation and differentiation of these cells, as well as promoting angiogenesis at injury sites[104]. Enhanced migratory and proliferative capacity and inhibited apoptosis of keratinocytes by activating the AKT/HIF-1α and Wnt/β-catenin pathways were observed with AMSC-Exos[129,130]. BMSC-Exos demonstrated the ability to promote fibroblast proliferation, migration, and secretion of growth factors and can induce tube formation in human umbilical vein cells (HUVECs)

[131]. AMSC-Exos induced angiogenesis in both *in vivo* and *in vitro* experiments, and the promotion of angiogenesis in endothelial cells was achieved by transferring miR-125a to inhibit DLL4 expression, accompanied by the downregulation of pro-angiogenic genes (Ang1 and Flk1), and upregulation of anti-angiogenic genes (Vash1 and TSP1)[132]. In addition to its pro-proliferative ability *in vitro*, the pro-healing effect of MSC-Exos has also been observed in acute non-diabetic wounds. MSC-Exos from human umbilical cord Wharton's jelly could regulate HaCaT cell function by suppressing AIF nucleus translocation and PARP-1 hyperactivation, thus attenuating full-thickness skin wounds by enhancing re-epithelialization and angiogenesis[133]. Fetal dermal-derived MSC-Exos accelerated wound closure in a mouse full-thickness skin wound model by activating the Notch signaling pathway to promote the motility and secretory capacity of fibroblasts[134].

Similarly, exosomes from MSCs improve proliferation and angiogenesis in diabetic wounds. AMSC-Exos accelerated cutaneous wound healing in diabetic mice with full-thickness skin wounds model by enhancing cell proliferation, inhibiting apoptosis, and promoting angiogenesis. They also repaired skin barrier functions, and produced large amounts, regular arrangement, and dense distribution of new collagen[123]. Shabbir *et al*[131] have also reported that these cells significantly increased their proliferation when treated with MSC-derived exosomes. Enhanced angiogenesis and fibroblasts proliferation, migration, and differentiation abilities were observed in diabetic wounds treated with human decidua derived MSC-Exos, as well as an improved fibroblast senescent state, reduced scar width, and larger and better-organized collagen deposition[135].

Various methods have been used to modify MSC-Exos to enhance fibroblast proliferation and angiogenesis. Co-culture of lncRNA H19-transfected BMSC-Exos with fibroblasts extracted from foot tissue of patients with DFUs revealed that overexpressed exosomes regulated the PTEN-mediated PI3K/AKT signaling pathway by competitively binding miR-152-3p to enhance proliferation and migration of fibroblasts and inhibit apoptosis and inflammation[136]. Injecting such exosomes into the peri-wound tissue of diabetic mice revealed the same changes in expression and accelerated wound healing[136]. Atorvastatin-pretreated BMSC-Exos promoted proliferation, migration of HUVECs, and vascular endothelial growth factor (VEGF) expression and accelerated wound healing in diabetic full-thickness skin injury rat models[137]. Pioglitazone-pretreated BMSC-Exos-treated full-thickness wounds in diabetic rats achieved faster-wound closure, with more adequate re-epithelialization and extensive collagen deposition, significantly enhanced wound perfusion, and had significantly upregulated levels of VEGF and CD31[138]. Subcutaneous injection of mmu_circ_0000250-modified AMSC-Exos *via* miR-128-3p/SIRT1-mediated autophagy promoted wound healing in diabetic mice, and increased capillary and granulation tissue production was detected owing to promoted proliferation and migration and reduced apoptosis of endothelial cells[139].

Biological scaffolds can improve the survival of exosomes in the inflammatory environment of diabetic wounds and maintain their sustained release. UCMSC-Exos combined with the Pluronic F127 hydrogel revealed promoted chronic wound healing in diabetic mice. The elevated number of blood vessels and microvascular density, enhanced regeneration of granulation tissue, and cell proliferation were also observed, with the significant formation of new hair follicles in the center of the wounds, sufficient subepidermal collagen deposition, and orderly arrangement of collagen fibers[140]. Similar changes were observed in the wounds of diabetic mice using engineered bioactive self-healing antimicrobial exosome hydrogels (FHE@exo), and the elevated number of dermal appendages and differentiation and re-epithelialization of the epidermis were also observed[141]. The combination of human gingival tissue-derived MSC-Exos (GMSC-Exos) and a chitosan/silk hydrogel sponge promoted re-epithelialization, angiogenesis, and collagen deposition, while the increased nerve fiber density also reflected enhanced neuronal ingrowth in the proliferative stage[142].

Matrix remodeling

In the final stage of wound healing, the production and remodeling of the ECM are key factors in determining the time of wound healing and degree of scarring. Recently, some studies have reported on the effects of exosomes on matrix remodeling. BMSC-Exos have been demonstrated to restore normal skin morphology in rats with full-thickness skin injury[143], while these capacities relied on the downregulation of TGF-β1 and upregulation of TGF-β3 by inhibiting the TGF-β/Smad signaling pathway. UCMSC-Exos had large amounts of miR-21, miR-23a, miR-125b, and miR-145, while it inhibited the differentiation and excessive aggregation of myofibroblasts and exerted an anti-scarring effect *via* the TGF-β2/Smad2 pathway *in vivo*[144]. UCMSC-Exos can also promote the phosphorylation of YAP, a key site of the Hippo pathway, to negatively regulate the Wnt4/β-catenin pathway to balance tissue regeneration and repair, with excessive cell proliferation and collagen deposition in the remodeling stage[145]. It was noted that intravenous injection of ADSC-Exos could increase the ratio of type III collagen to type I and TGF-β3 to TGF-β1, prevent fibroblast-to-myofibroblast differentiation, and reduce scarring at incisions in the full-thickness skin injury models[146]. They could also induce the ERK/MAPK pathway in fibroblasts to increase the expression of MMP3, thereby increasing MMP3/TIMP1 to regulate ECM remodeling[146].

In contrast to the promoted cell proliferation and abundant granulation tissue in the early stage of healing, proliferative activities were reduced during the late repair stage to prohibit tissue hyperplasia when using FHE@exo, suggesting entry into the remodeling phase that prevents excessive tissue prolif-

eration to promote wound healing[141]. The application of GMSC-Exos with chitosan/silk hydrogel sponge on the wounds of diabetic rats revealed more collagen deposition and thick wavy collagen fibers that were arranged in an orderly fashion, which is similar to that in normal skin, implying enhanced ECM remodeling[142]. These were also observed in the local transplantation of HUCMSC-Exos with polyvinyl alcohol/alginate nano hydrogel and of miR-126-3p overexpressed synovial-derived MSC-Exos with hydroxyapatite/chitosan composite hydrogel[147,148]. Altogether, these studies indicate that MSC-Exos play a pivotal role in the ECM remodeling phase of wound healing.

The various stages of wound healing are closely interwoven. MSC-Exos inherit the genetic information of their parental cells and can transfer the therapeutic bioactive substances to target cells to participate in intercellular communication, resulting in the regulation of target cell function and promotion of wound healing[81,149]. We analyzed the current preclinical application of MSC-Exos in diabetic wound models, and the cell source, administration method, dose, frequency, animal type, wound diameter, efficacy, and possible molecular mechanisms are summarized in Table 1[104,121,123-128,147,148,135-142,150-158]. Additionally, MSC-Exos were not only responsible for a specific stage but also promote microenvironment changes in the wounds at each stage to exert a pro-healing effect. Although the biological functions of promoting diabetic wound healing are generally similar, certain differences exist in the regulated signaling pathways of different cell-derived exosomes or receiving different preconditioning, according to previous studies. The regulatory mechanisms most frequently studied in diabetic wound models and may potentially confirmed in DCWs, as well as the microenvironmental changes in inflammatory and proliferative stages of wound healing after using MSC-Exos, are depicted in Figure 1.

CURRENT STATUS AND PROSPECTS OF CLINICAL APPLICATIONS OF EXOSOMES IN DCWS

Preclinical studies have demonstrated the ability of MSC-Exos to promote diabetic wound healing. No evident pathological abnormalities in the heart, liver, spleen, lung, and kidneys sampled after exosome treatment were observed, and biomarkers reflecting liver and kidney function blood biochemistry were also within normal limits[127]. Meanwhile, no erythema, edema, or irritation was observed in the wound area after exosome treatment[137], confirming the superior biosafety of exosome therapy.

We also searched for applications of exosomes secreted by stem cells from other sources in diabetic wounds and summarized them in Supplementary Table 1. Noteworthy, the types of animals used for modeling were limited to mice and rats. Most of the studies involved acute diabetic wounds, that is, exosomes were administered immediately after successful modeling of full-thickness skin wounds. Only one study introduced *Staphylococcus aureus* to establish infected chronic wounds after the establishment of full-thickness cutaneous wounds and confirmed that exosomes were effective in treating infectious DCWs[127]. The efficacy and safety of MSC-Exos need to be further confirmed in larger animal models and DCW models. Because the islet morphology, structure and function, blood biochemical indices, and skin structure of minipigs are more similar to those of the human body, they are ideal animal models for studying diabetic wounds[159]. Our team has established a chronic skin ulcer model in diabetic miniature pigs in the early stage[160] and is researching on exosome products to explore the optimal administration methods and dosages and to verify their therapeutic effects.

According to the search results in ClinicalTrials.gov, no clinical trials of MSC-Exos and exosomes from other sources for diabetic cutaneous wound healing have been registered. Therefore, we expanded the scope of clinical trials to search for exosomes derived from any sources and exosome-enriched stem cell-conditioned medium in various wound types (Table 2). None of the included four registered clinical trials had related results published, while they were all non-randomized one-arm pilot studies. Thus, more high-quality randomized controlled trials are required to further confirm these research results. Of note, the application of cell-free therapies in clinical patients requires special attention to security, although no adverse reactions of exosomes have been reported in preclinical studies. Moreover, ADSC-Exos has been confirmed to not induce any irritation or toxicity in skin sensitization, irritation, or oral toxicity tests[161]; therefore, they can be considered in clinical practice to promote wound healing in combination with basic wound care measures. Nevertheless, toxicological analysis of different tissue-derived MSCs-Exos and more evidence of short and long-term health safety assessments are required to confirm their safety.

Exosome research is still in its infancy, and the realization of the transformation from preclinical research to clinical application still has great exploration value. The problems of optimal preparation, extraction, isolation, and storage of exosomes on a large scale and their production efficiency have not yet been determined; preparation and identification of components due to different source cells and the high heterogeneity of exosome components have not yet been solved; specific regulatory mechanisms in DCWs have not yet been fully elucidated; efficacy and safety of different cell sources and/or administrations have not been proven, and reasonable and effective methods of fusing exosomes with other biomaterials have not yet been implemented, all these issues are barriers that limit the clinical application of exosomes.

Table 1 Mesenchymal stem cell-derived exosomes application of diabetic full-thickness acute/chronic cutaneous wounds model

No.	Ref.	Institution(Nation)	Exosomes source	Intervention, administration, dose and time	Control	Model species	Wound diameter	Therapeutic effect	Molecular mechanism
1	Yang *et al*[140], 2020	The Third Affiliated Hospital of Southern Medical University(China)	Human umbilical cord	1 HUCMSC-Exos + PF-127 hydrogel; injected topically; 100 μg in 100 μL PF-127 (24%); at Day 0 2 HUCMSC-Exos + PF-127 hydrogel; injected topically; 100 μg in 100 μL PBS; at Day 0 3 PF-127 hydrogel; injected topically; 100 μL PF-127 (24%); at Day 0	PBS (100 μL)	Rats (Sprague-Dawley)	10 mm × 2 (1.5 cm apart)	1 Accelerated wound closure rate 2 New hair follicle formation, fibroblasts proliferation, sufficient and order collagen deposition 3 Reduced inflammatory cell infiltration 4 Higher microvessel densities and higher number of blood vessels (CD31, MVD) 5 Promoted cell proliferation (Ki67) and enhanced regeneration of granulation tissue 6 Upregulated expression of VEGF and TGF-β 7 Hydrogel supported exosome survival and biological activity	–
2	Wang *et al*[141], 2019	The Affiliated Hospital of Wenzhou Medical University; Xi'an Jiaotong University(China)	Mouse adipose tissue	1 AMSC-Exos + F127/OHA-EPL hydrogel; covered the wound; 10 μg; at Day 0 2 AMSC-Exos; covered the wound; 10 μg; at Day 0 3 F127/OHA-EPL hydrogel; covered the wound; 10 μg; at Day 0	Saline	Mice (ICR)	8 mm × 2 mm	1 Accelerated wound closure rates 2 Promoted cell proliferation and abundant granulation tissue in early stage of healing; reduced proliferative activities during the late repair stage to prohibit tissue hyperplasia 3 Abundant and well-organized collagen fibers, more collagen deposition (Col I, Col III) 4 Faster re-epithelization (cytokeratin) and epithelial cell differentiation	–

								5 Promoted angiogenesis (α-SMA) and blood vessels formation 6 Complete skin regeneration: skin appendages and less scar tissue appeared	
3	Liu *et al*[121], 2020	Second Military Medical University; Shanghai Sixth People's Hospital affiliated to Shanghai Jiao Tong University(China)	Human bone marrow	1 Melatonin-pretreated BMSC-Exos (MT-Exo); injected subcutaneously at least six sites per wound; dose not mentioned; at Day 0	PBS	Rats (Sprague-Dawley)	20 mm	1 Accelerated diabetic wound healing 2 Anti-inflammatory effect on macrophages by promoting M2 and inhibiting M1 polarization 3 Enhanced re-epithelialization (increased neoepithelium length) 4 Improved angiogenesis (α-SMA, CD31, Microfli perfusion) and collagen synthesis (Col I and III) 5 Activated the PTEN/AKT signaling pathway	PTEN/AKT signaling pathway
				2 BMSC-Exos; injected subcutaneously at least six sites per wound; dose not mentioned; at Day 0					
4	Pomatto *et al* [104], 2021	University of Turin(Italy)	Human bone marrow	BMSC-EVs + carboxymethylcellulose; applied on the wound; 1 × 10^9 in 25 μL of vehicle; at Day 0, 3, 7 and 10	carboxymethylcellulose high viscosity 10 mg/mL (25 μL)	Mice (NSG)	6 mm × 8 mm	Not effective and did not reduce the wound closure rate	–
			Human adipose tissue	AMSC-EVs + carboxymethylcellulose; applied on the wound; 1 × 10^9 in 25 μL of vehicle; at Day 0, 3, 7, 10 and 14				1 Accelerated cutaneous wound healing 2 Reduced size of the scar 3 Increased epithelial thickness and re-epithelization 4 Promoted angiogenesis (the number of vessels)	
5	Shi *et al*[139], 2020	Affiliated Hospital of Nantong university(China)	Human adipose tissue	1 mmu_circ_0000250-modified AMSC-Exos;injected subcutaneously at four sites around the wound;200 μg in 100 μL PBS;at Day 0	PBS (100 μL)	Mice (C57BL)	4 mm	1 Accelerated cutaneous wound healing 2 Reduced scar areas 3 Enhanced angiogenesis	mmu_circ_0000250/miR-128-3p/SIRT1-mediated autophagy

							(CD31, vessel density) 4 Suppressed apoptosis of skin tissue 5 Suppressed expression of miR-128-3p but promoted SIRT1 expression 6 Increased expression of autophagy-related gene (LC3)	
				2 AMSC-Exos; injected subcutaneously at four sites around the wound; 200 μg in 100 μL PBS; at Day 0				
6	Hu *et al*[138], 2021	Union Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology(China)	Rat bone marrow	1 Pioglitazone-treated BMSC-Exos (PGZ-Exos); injected subcutaneously(at least six sites per wound); 100 μg in 100 μL PBS; at Day 0	PBS (100 μL)	Rats (Sprague-Dawley)	15 mm	1 Accelerated cutaneous wound healing 2 Enhanced re-epithelization 3 Promoted collagen synthesis (Col I, Col III) and collagen deposition, indicating more superior ECM remodeling ability 4 Enhanced angiogenesis (VEGF, CD31) and blood flow of the wound	PTEN/PI3K/AKT/eNOS pathway
				2 BMSC-Exos; injected subcutaneously (at least six sites per wound); 100 μg in 100 μL PBS; at Day 0				
7	Yu *et al*[137], 2020	Shanghai Sixth People's Hospital affiliated to Shanghai Jiao Tong University; Second Military Medical University(China)	Human bone marrow	1 Atorvastatin-pretreated BMSC-Exos (ATV-Exos); injected subcutaneously (six points); dose not mentioned; at Day 0 2 BMSC-Exos; injected subcutaneously (six points); dose not mentioned; at Day 0	PBS	Rats (Sprague-Dawley)	20 mm	1 Accelerated cutaneous wound healing 2 Increased re-epithelization (more epithelial structures and longer neuroepithelium) 3 Promoted collagen synthesis and deposition, indicating more superior ECM remodeling ability (thicker wavy collagen fibers and more extensive collagen deposition arranged neatly) 4 Superior biosafety of the therapy of exosomes 5 Enhanced angiogenesis (CD31, α-SMA and Microfil perfusion)	miR-221-3p /PTEN/AKT/eNOS pathway

8	Zhao *et al*[123], 2021	Tongji University(China)	Human adipose tissue	1. AMSC-Exos; smeared at the wound; 200 μg in 200 μL PBS; 3 times/day, 2 wk 2 Recombinant human epidermal growth factor (rhEGF); smeared at the wound;3 times/day, 2 wk 3 AMSC-CM; smeared at the wound; 3 times/day, 2 wk	PBS;Untreated	Mice (db/db)	15 mm	1 Accelerated cutaneous wound healing 2 Exosomes entered the dermis of wounds after smearing 3 Mild hyperkeratosis and typical fibrous structures with new glands and hair follicles, implying enhanced tissue remodeling 4 Enhanced collagen synthesis (Col I, Col III), deposition and remodeling (large amounts, large area, regular arrangement and dense distribution of new collagen) 5 Enhanced cell proliferation and inhibited apoptosis 6 Increased blood vessel intensity and promoted angiogenesis (CD31, VEGF) 7 Repaired skin barrier functions (elevated expression levels Filaggrin, Loricrin, and AQP3) 8 Suppressed expression of inflammatory cytokines (IL-6, TNF-α, CD14, CD19 and CD68) 9 Negatively regulated MMP1 and MMP3 expression in promoting collagen synthesis	–
9	Tao *et al*[150], 2017	Shanghai Jiao Tong University Affiliated Sixth People's Hospital(China)	Human synovial membrane	1 miR-126-3p overexpressed SMSC-Exos + chitosan wound dressings; placed on the wound bed with pressure dressing; at Day 0 2 Chitosan wound dressings;	Untreated	Rats (Sprague-Dawley)	18 mm	1 Accelerated cutaneous wound healing 2 Enhanced angiogenesis (microcomputed tomography, CD31, α-SMA) 3 Promoted re-epithelial-	PI3K/AKT and MAPK/ERK signaling pathways

				placed on the wound bed with pressure dressing; at Day 0				ization, granulation tissue formation, collagen alignment and deposition, implying enhanced ECM remodeling 4 Accelerated development of hair follicles and sebaceous glands	
10	Ti *et al*[126], 2015	Chinese PLA General Hospital(China)	Human umbilical cord	1 LPS-pretreated HUCMSC-Exos; injected dispersively into the wound edge; 60 μg in 0.5 mL PBS; at Day 0 2 HUCMSC-Exos; injected dispersively into the wound edge; 60 μg in 0.5 mL PBS; at Day 0	Untreated	Rats	10 mm	1 Accelerated cutaneous wound healing 2 Decreased inflammatory cell infiltration 3 Regulate macrophage polarization to M2 macrophages 4 Promoted the appearance of new small capillaries	let-7b/TLR4/NF-κB/STAT3/AKT pathway
11	Li *et al*[136], 2020	The Fourth Affiliated Hospital of Harbin Medical University(China)	Mouse bone marrow	1 lncRNA H19 overexpressed BMSC-Exos; injected into the skin around the wound; at Day 0 2 BMSC-Exos; injected into the skin around the wound; at Day 0	Untreated	Mice (C57BL/6)	10 mm	1 Accelerated cutaneous wound healing. 2 Ameliorated inflammation of the wound (IL-10↑, IL-1β↓, TNF-α↓ and fewer inflammatory cells around the wound) 3 Promoted granulation tissue formation 4 Enhanced angiogenesis (Increased expression of VEGF, TGF-β1, α-SMA, and Col I) 5 Suppressed cell apoptosis 6 Interacted with miR-152-3p *via* PTEN-mediated PI3K/AKT signaling pathway (diminished miR-152-3p expression, elevated PTEN expression and decreased expression of PI3K, AKT and p-AKT)	lncRNA H19/miR-152-3p/PTEN/ PI3K/AKT signaling pathway
12	Shi *et al*.(2017) [142]	Chinese PLA General Hospital(China)	Human gingival tissue	1 GMSC-Exos+ chitosan/silk hydrogel sponge; covered the wound with restraining	1. PBS (100 μL);2. gauze (13 mm× 13 mm) covered the wound	Rats (Sprague-Dawley)	10 mm	1 Accelerated cutaneous wound healing	—

				bandage; 150 μg in 100 μl PBS; at Day 0, changed every 3 d 2 Chitosan/silk hydrogel sponge; covered the wound with restraining bandage; in 100 μL PBS; at Day 0, changed every 3 d				2 Promoted re-epithelialization, deposition and remodeling of ECM (more collagen deposition and thick wavy collagen fibers, the collagen fibers arranged in an orderly fashion similar to that of normal skin) 3 Enhanced angiogenesis (CD34, microvessel density) 4 Enhanced neuronal ingrowth (nerve fiber density)	
13	Xiao *et al*[151], 2021	Nan Fang Hospital of Southern Medical University(China)	Human adipose tissue	1 AMSC-Exos + human acellular amniotic membrane (hAAM) scaffold; covered on the wound; 100 μg in 100 μL PBS; at Day 0, every other day, 3 times in total 2 AMSC-Exos; covered on the wound;100 μg in 100 μL PBS; at Day 0, every other day, 3 times in total 3 hAAM patch; covered on the wound; at Day 0, every other day, 3 times in total	PBS (100 μL)	Mice (BALB/c)	10 mm	1 Accelerated cutaneous wound healing 2 Suppressed wound inflammatory responses (fewer inflammatory cells around the wound and higher recruitment of M2 macrophages to the wound sites) 3 Enhanced angiogenesis (CD31) 4 Enhanced extracellular matrix (ECM) deposition (Col III) 5 Promoted re-epithelialization (completed epithelial and dermal regenerated) 6 Failed regenerated hair follicle and sebaceous glands	–
14	Yan *et al*[152], 2022	Union Hospital, Tongji Medical College, Huazhong University of Science and Technology(China)	Human umbilical cord	1 HUCMSC-Exos injected locally to the wound site; 100 μL, 50 μg/ml; at days 0, 3, 5, 7, 9, and 11 2 HUCMSC-Exos injected locally to the wound site; 100 μL, 100 μg/mL; at days 0, 3, 5, 7, 9, and	PBS (100 μL)	Mice (C57BL/6J)	10 mm	1 Accelerated cutaneous wound healing 2 Reduced oxidative stress (ROS) 3 Promoted granulation tissue formation	–

				11				4 Enhanced angiogenesis (CD31, mean perfusion unit ratio)	
15	Geng *et al*[128], 2022	Jinzhou Medical University(China)	Rat bone marrow	1 BMSC-Exos + carboxyethyl chitosan-dialdehyde carboxy-methyl cellulose hydrogel; covered the wound; twice a day, two weeks 2 Carboxyethyl chitosan-dialdehyde carboxymethyl cellulose hydrogel; covered the wound; twice a day, two weeks	Untreated	Rats (Sprague-Dawley)	20 mm	1 Accelerated cutaneous wound healing 2 Promoted collagen deposition and remodeling, and fibrin regeneration 3 Enhanced antibacterial effects by significantly inhibiting bacterial growth 4 Skew macrophage functional polarity from M1 (iNOS) towards an anti-inflammatory M2 phenotype (CD206) 5 Decreased inflammatory factors (IL-1β, TNF-α) 6 Promoted proliferation of blood vessels and angiogenesis (CD31)	VEGF-mediated PI3K/AKT signaling pathways
16	Gondaliya *et al* [153], 2022	National Institute of Pharmaceutical Educationand Research(India)	Bone marrow	1 BMSC-Exos loaded with miR-155 inhibitor; injected subcutaneously; 0.1 μg/μL; 1 d after wound induction 2 BMSC-Exos; injected subcutaneously; 0.1 μg/μL; 1 d after wound induction 3 BMSC-Exos loaded with negative control sequences; injected subcutaneously; 0.1 μg/μL; 1 d after wound induction	Untreated	Mice (C57BL/6)	4 mm	1 Accelerated cutaneous wound healing 2 Declined miR-155 levels with a concomitant increase in FGF-7 3 Downregulated expression of MMP-2 and MMP-9 4 Declined expression of pro-inflammatory cytokines (TIMP-2, lymphotactin, sTNF RI, sTNF RII, and LIX); declined regulated upon activation, normal T cell expressed and secreted (RANTES) chemokine; downregulated pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) and TGF-β1 5 Promoted re-epithelialization, collagen synthesis	–

								and deposition, angiogenesis (α-SMA) and vascularization (CAM)	
17	Dalirfardouei *et al*[125], 2019	Mashhad University of Medical Sciences(Iran)	Human menstrual blood	1 MenSC-Exos; injected intradermally; 10 μg in 100 μL of PBS; at Day 0 2 MenSCs; injected intradermally; 1 × 10^6 cells in 100 μL of PBS; at Day 0	PBS (100 μL)	Mice (C57BL/6)	8 mm	1 Accelerated cutaneous wound healing 2 Promoted re-epithelialization 3 Induced macrophage polarization from M1 (iNOS) to M2 (Arg) phenotype 4 Enhanced angiogenesis (VEGF, microvessel density) 5 Improved collagen deposition (upregulated Col I/Col III ratio at Day 7, downregulated at Day 14) 6 Decreased size of scar tissues 7 Decreased cellularity in the granulation tissue 8 Decreased *Rela* gene expression at Day 4, enhanced at Day 7.	NF-κB signaling pathway (possible)
18	Wang *et al*[124], 2022	Affiliated Hospital of Nantong University(China)	Rat bone marrow	1 BMSC-Exos + 50 mg/kg intraperitoneal tertbutylhydroquinone (tBHQ); injected subcutaneously of 4 sites at the base and edge of the wound; 100 μg/mL, 200 μL; at Day 0 and 7 2 BMSC-Exos + 200 μL intravenous Lenti-sh-NC; injected subcutaneously of 4 sites at the base and edge of the wound; 100 μg/mL, 200 μL; at Day 0 and 7 3 BMSC-Exos; injected subcutaneously of 4 sites at the base and edge of the wound; 100	PBS	Rats (Sprague-Dawley)	15 mm	1 Accelerated cutaneous wound healing 2 Promoted re-epithelialization and collagen deposition 3 Enhanced angiogenesis (CD31) 4 Reduced inflammation (decreased inflammatory cytokines TNF-α, IL-1β and increased anti-inflammatory cytokines IL-4, IL-10).	–

				μg/mL, 200 μL; at Day 0 and 7 4 BMSC-Exos + 200 μL intravenous Lenti-sh-Nrf2; injected subcutaneously of 4 sites at the base and edge of the wound; 100 μg/mL, 200 μL; at Day 0 and 7					
19	Sun *et al*[127], 2022	Nanjing Normal University; Nanjing University; Nanjing medical University; Nanjing Tech University(China)	Human umbilical vein	1 Engineering TNF-α/hypoxia-pretreated HUVMSC-Exos +PCOF; each subsequent day later, total 21 d 2 Engineering TNF-α/hypoxia-pretreated HUVMSC-Exos; each subsequent day later, total 21 d 3 Vancomycin; each subsequent day later, total 21 d 4 PCOF; each subsequent day later, total 21 d	PBS		Mice (C57BL/6)	15 mm (*S.aureus*-infected chronic wounds)	1 Accelerated cutaneous wound healing 2 Reduced bacterial burden and suppressed bacterial colonization in the wound sites 3 Reduced the inflammatory response (immune cells counting); decreased proinflammatory cytokines (TNF-α, IL-1β, IL-6); induced M2 (CD206) macrophages polarization 4 Promoted collagen deposition and remodeling, granulation formation, re-epithelialization and enhanced proliferation of fibroblasts 5 Enhanced cell proliferation (Ki67) 6 Suppressed oxidative stress induced by bacteria and peroxide substrates (reduced the content of oxidative biomarkers and (MDA) increased the antioxidant mediators (GSH-Px, SOD) 7 Promoted angiogenesis (upregulated miR-126, HIF-1α, VEGF, CD31 and α-SMA; increased neovascularization) 8 *In vivo* biosafety (blood system, heart, liver, kidney and other organs)	miR-126/SPRED1/RAS/ERK pathway (possible)

20	Li *et al*[147], 2016	Shanghai Normal University; Shanghai Jiao Tong University Affiliated Sixth People's Hospital(China)	Human synovial tissue	1 miR-126-3p overexpressed SMSC-Exos + hydroxyapatite/chitosan composite hydrogel; placed on the wound bed with pressure dressing 2 Hydroxyapatite/chitosan composite hydrogel; placed on the wound bed with pressure dressing	Untreated	Rats (Sprague-Dawley)	18 mm	1 Accelerated cutaneous wound healing 2 Enhanced angiogenesis (μCT), formation and maturation of new vessels (CD31, α-SMA) 3 Promoted re-epithelialization, granulation tissue maturation, collagen alignment and deposition that indicated improved ECM remodeling 4 Accelerated growth of follicles and sebaceous glands	Activated MAPK/ERK and PI3K/AKT pathways
21	Zhang *et al*[148], 2021	Jinzhou Medical University(China)	Human umbilical cord	1 HUCMSC-Exos + polyvinyl alcohol (PVA)/alginate (Alg) nanohydrogel; locally transplanted; 300 μL; once a day 2 HUCMSC-Exos; locally transplanted; 300 μL; once a day 3 PVA/Alg nanohydrogel; locally transplanted; 300 μL; once a day	Untreated	Rats (Sprague-Dawley)	15 mm × 2 mm	1 Accelerated cutaneous wound healing 2 Enhanced re-epithelialization and hair follicles formation 3 Promoted collagen deposition and remodeling (increased and orderly arranged collagen fibers) 4 Promoted angiogenesis (CD31, α-SMA, SR-B1, VEGF)	ERK1/2 pathway
22	Han *et al*[154], 2022	The First Affiliated Hospital of Zhengzhou University(China)	Human bone marrow	1 lncRNA KLF3-AS1 overexpressed BMSC-Exos; injected *via* tail vein; 100 μL; at Day 0 2 Negative control silenced BMSC-Exos;injected *via* tail vein;100 μL;at Day 0 3 Negative control overexpressed BMSC-Exos; injected *via* tail vein; 100 μL; at Day 0 4 lncRNA KLF3-AS1 silenced BMSC-Exos; injected *via* tail vein; 100 μL; at Day 0	Untreated	Mice (BALB/c)	Not mentioned	1 Accelerated cutaneous wound healing 2 Minimized weight loss. 3 Reduced inflammation (decreased IL-6 and IL-1β) 4 Promoted angiogenesis (CD31), collagen deposition and follicle regeneration 5 Decreased expression of miR-383 and increased VEGFA	lncRNA KLF3-AS1/miR-383/VEGFA signaling pathway
23	Ding *et al*[155], 2019	Shanghai Jiao Tong University Affiliated	Human bone marrow	1 Deferoxamine-preconditioned BMSC-Exos (DFO-Exos); injected	PBS (100 μL)	Rats (Sprague-Dawley)	20 mm × 2 mm	1 Accelerated cutaneous wound healing	miR-126/PTEN/PI3K/AKT pathway

		Sixth People's Hospital(China)		subcutaneously around the wounds at four sites; 100 μg in 100 μL PBS; at Day 0 2 BMSC-Exos; injected subcutaneously around the wounds at four sites; 100μg in 100μL PBS; at Day 0				2 Enhanced re-epithelialization and lower scar formation 3 Promoted collagen deposition (increased wavy collagen fibers) 4 Promoted angiogenesis (vessel density by micro-CT, CD31, α-SMA)	
24	Bian *et al*[135], 2020	Chinese PLA General Hospital(China)	Human decidua	dMSC-sEVs; injected around the wounds at 4 sites (25 μL per site); 100 μL, 5.22×10^{11} particles/mL; at Day 7, 14, 21and 28	PBS (100 μL)	Mice (BKS-db)	16 mm	1 Accelerated cutaneous wound healing 2 Reduced scar width 3 Accelerated collagen deposition (larger and better-organized collagen deposition) 4 Enhanced fibroblast proliferation (PCNA), migration (CXCR4), and differentiation abilities of fibroblast 5 Promoted angiogenesis (α-SMA) 6 Improved fibroblast senescent state (p21)	RAGE/RAS; Smad pathways
25	Zhang *et al*[156], 2022	Xijing Hospital of Fourth Military Medical University(China)	Human adipose tissue	AMSC-Exos; injected subcutaneously; 200 μg; 3 d after wound induction, for three consecutive days	PBS (100 μL)	Mice (db/db)	10 mm	1 Accelerated cutaneous wound healing 2 Enhanced re-epithelialization 3 Promoted angiogenesis (CD34, VEGF) 4 Improved oxidative stress (MDA, T-AOC, SOD) 5 Reduced inflammatory cytokines (IL-1β, IL-6, TNF-α, MCP-1)	SIRT3/SOD2 pathway
26	Born *et al*[157], 2021	University of Maryland; Johns Hopkins University School of Medicine(USA)	Human bone marrow	1 HOX transcript antisense RNA (HOTAIR) overexpressed BMSC-EVs; injected around the wound in a cross pattern of four	PBS (50 μL)	Mice (db/db)	8 mm	1 Accelerated cutaneous wound healing 2 Promoted angiogenesis	–

				sites; 50 μg in 50 μL PBS; at Day 3, four times 2 BMSC-EVs; injected around the wound in a cross pattern of four sites; 50 μg in 50 μL PBS; at Day 3, four times				(CD31, VEGFA)	
27	Teng *et al*[158], 2022	Jiangnan University (China)	Human umbilical cord	HUCMSC-Exos; injected subcutaneously around the wounds at four sites; 100 μL (100 μg/mL); at Day 0	PBS (100 μL)	Rats (Sprague-Dawley)	10 mm	1 Accelerated cutaneous wound healing 2 Inhibited chronic inflammation: (decreased number of inflammatory cells); inhibited pro-inflammatory cytokines (TNF-α); induced M2 (CD206) macrophages polarization 3 Enhanced re-epithelialization 4 Promoted angiogenesis (increased new blood vessels, CD31, VEGF) 5 Promoted collagen synthesis and skin regeneration	–

HUCMSC-Exos: Human umbilical cord mesenchymal stem cell derived exosomes; PF-127: Pluronic F-127; PBS: Phosphate buffered saline; MVD: Microvascular density; Ki67: Nucleus related antigen; TGF-β: Transforming growth factor-β; VEGF: Vascular endothelial growth factor; F127: Pluronic F127; OHA: Oxidative hyaluronic acid; EPL: Poly-ε-L-lysine; Col I: Collagen I; Col III: Collagen III; α-SMA: Alpha smooth muscle actin; BMSC-Exos: Bone marrow mesenchymal stem cell derived exosomes; PTEN: Phosphatase and tensin homolog; BMSC-EVs: Bone marrow mesenchymal stem cell derived extracellular vesicles; AMSC-EVs: Adipose tissue mesenchymal stem cell derived extracellular vesicles; AMSC-Exos: Adipose tissue mesenchymal stem cell derived exosomes; SIRT1: Silent mating type information regulation 2 homolog-1; LG3: Light chain 3; ECM: Extracellular matrix; PI3K: Phophatidylinositol3-kinase; eNOS: Endothelial nitric oxide synthase; AMSC-CM: Adipose tissue stem cell conditioned medium; AQP3: Recombinant aquaporin 3; IL-6: Interleukin 6; TNF-α: Tumor necrosis factor alpha; SMSC-Exos: Synovial membrane mesenchymal stem cell derived exosomes; MAPK: Mitogen-activated protein; ERK: Extracellular signal regulated kinase; let-7b: MicroRNA let-7b; TLR4: Toll like receptor 4; NF-κB: Nuclear factor kappa-B; STAT3: Signal transducer and activator of transcription 3; Il-10: Interleukin 10; IL-1β: Interleukin 1β; GMSC-Exos: Gingival tissue mesenchymal stem cell derived exosomes; iNOS: Inducible nitric oxide synthase; sTNF RI: Soluble tumor necrosis factor receptor I; sTNF RII: Soluble tumor necrosis factor receptor II; FGF-7: Fibroblast growth factor 7; LIX: Lipopolysaccharide-induced CXC chemokine; CAM: Chick chorioallantois membrane; MenSC-Exos: Menstrual blood mesenchymal stem cell derived exosomes; MenSCs: Menstrual blood-derived mesenchymal stem cells; Arg: Arginase; Lenti-sh-Nrf2: Lentiviral shRNA targeting Nrf2; Lenti-sh-NC: Lentiviral control shRNA; HUVMSC-Exos: Human umbilical vein mesenchymal stem cell derived exosomes; PCOF: Polydopamine modified reductive covalent organic frameworks; *S.aureus*: *Staphylococcus aureus*; MDA: Malondialdehyde; GSH-Px: Glutathione peroxidase; SOD: Superoxide dismutase; SR-B1: Scavenger receptor class B type I; dMSC-sEVs: Decidua mesenchymal stem cell derived extracellular vesicles; PCNA: Proliferating cell nuclear antigen; CXCR4: CXC-chemokine receptor 4; p21: Cyclin-dependent kinase inhibitor 1A; RAGE: Receptor for advanced glycation end products; RAS: rat sarcoma; T-AOC: Total antioxidant capacity; ,MCP-1: Monocyte chemoattractant protein-1; SIRT3: Silent mating type information regulation 2 homolog 3.

Thus, efficient, stable, safe, and mass-producible stem cells and related products for the treatment of diabetic wounds are yet to be explored and developed. More research is required in future clinical trials and routine practice to determine the most effective cell sources for diabetic wounds; to establish optimal large-scale culture conditions of MSCs; to solve the preparation problem of huge heterogeneity of exosome components; to explore standardized isolation, quality control, purification, and characterization techniques of MSC-Exos; and to determine the best approach for long-term storage[162].

Table 2 Clinical trials of exosomes in treating various wounds

Start year	Institution (Nation)	Type of wounds	Intervention	Autologous/Allogeneic	Administration, frequency	Patients number	Follow-up period	Outcome measures	Phase	Study design	ClinicalTrials.gov identifier	Status
2022	Shanghai Ninth People's Hospital Affiliated to Shanghai Jiao Tong University (China)	Full-layer skin wounds	Adipose tissue derived exosomes(200-300 mL of the subject adipose tissue)	Autologous	Applied directly to the wound (mixed with sterile hydrogel), twice a week	5	4 wk	**Primary:** Percentage of wound healing	Not Applicable	Non-randomized, single group assignment, open label	NCT05475418	Not yet recruiting
2015	Kumamoto University (Japan)	Intractable cutaneous ulcers (*e.g.*, rheumatic disease, peripheral arterial disease, chronic venous insufficiency, decubitus or burns)	Plasma-derived exosomes (Plasma samples will be filtered through 0.45 μm and 0.20 μm filters. The samples will be filtered through 0.02 μm filter to trap exosomes with the filter. Saline solution will be loaded from the other side of the 0.02 μm filter to obtain exosome rich buffer.)	Autologous	Applied to the ulcer, daily	5	28 d	**Primary:** Ulcer size (length, width, depth) **Secondary:** Pain of cutaneous wounds (VAS)	Early Phase 1	Non-randomized, single group assignment, open label	NCT02565264	Unknown
2023	Aegle Therapeutics (USA)	Dystrophic Epidermolysis Bullosa (DEB); chronic wounds (< 20% closure of wound during observation period); 10-50 cm^2	Bone marrow mesenchymal stem cells derived extracellular vesicle (AGLE-102)	Allogeneic	Multiple administrations of 2 ascending dose levels of AGLE-102; (up to 6 administrations); (each administration will occur 14 ± 7 d but no less than 7 d apart); (each administration no more than 3 mo); (wound closes prior to 6 administrations, no additional doses will be given)	10	8 mo; if the wound closes before receiving all 6 doses, for 4 mo after the wound closes	**Primary:** Dose limiting toxicity **Secondary:** Wound size	Phase 1/2	Non-randomized, multicenter, ascending dose, single group assignment, open label	NCT04173650	Not yet recruiting
2019	Mayapada Hospital (Indonesia)	Chronic wounds	Human Wharton's Jelly mesenchymal stem cells conditioned medium (WJ-MSC-CM)	Allogeneic	Applied to the wound (the conditioned medium gel), every week	38	2 wk	**Primary:** Success rate of chronic ulcer healing	Phase 1	Non-randomized, single group assignment, open label	NCT04134676	Completed

Researchers also need to fully understand the abilities, loss, distribution, diffusion efficiency, and clearance efficiency of exosomes after transporting them to target areas. Physical, chemical, or biological methods for preconditioning, genetic engineering, and transfection are used to specifically enhance a certain therapeutic potential to achieve relatively better wound healing than native exosomes, thus becoming new treatment directions[163]. Additionally, combining exosomes with biomaterials is

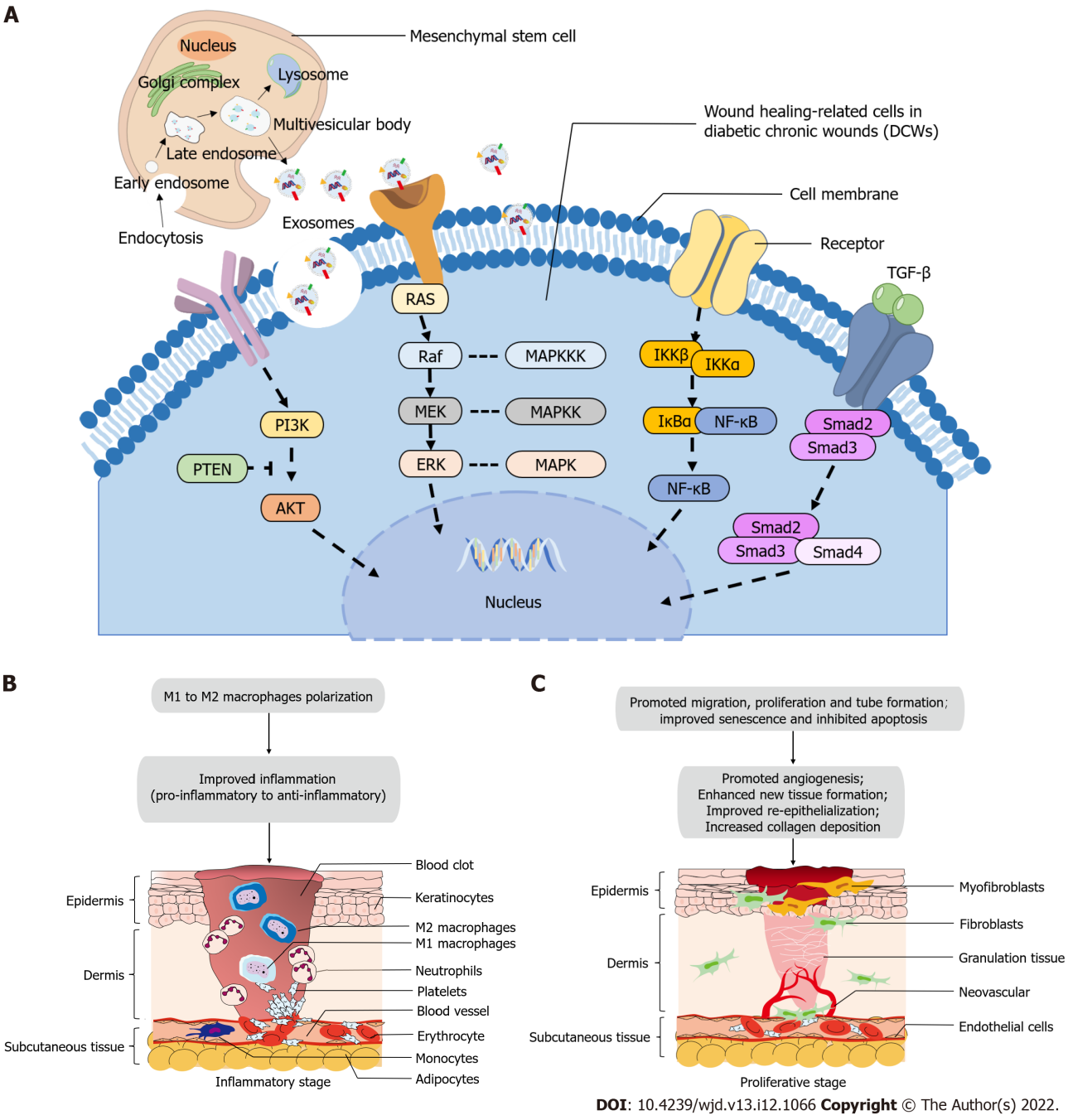

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Figure 1 Molecular mechanism of mesenchymal stem cell-derived exosomes in diabetic cutaneous wound healing. A: signaling pathways most frequently studied in diabetic wound models and may potentially confirmed in diabetic chronic wounds; B: microenvironmental changes in inflammatory stage of wound healing after using mesenchymal stem cell-derived exosomes; C: microenvironmental changes in proliferative stage of wound healing after using mesenchymal stem cell-derived exosomes. PTEN: Phosphatase and tensin homolog; PI3K: Phophatidylinositol3-kinase; Akt/PKB: Protein kinase B; RAS: Rat sarcoma; Raf: Rapidly accelerated fibrosarcoma; MAPK: Mitogen-activated protein; ERK: Extracellular signal regulated kinase; NF-κB: Nuclear factor kappa-B; TGF-β: Transforming growth factor-β; Smad2/3/4: Drosophila mothers against decapentaplegic.

possible to create bioactive dressings to enhance or combine repair ability, provide local microenvironment stability, and achieve sustained release of exosomes[74]. Additionally, starting clinical trials as soon as possible is necessary to verify the optimal dosages, administration methods, and efficacy evaluation of MSC-Exos in clinical patients, looking forward to its broad application prospects in promoting DCW healing in clinical practice[162].

CONCLUSION

DCWs, which are one of the most common chronic refractory wounds, pose a heavy burden to patients, families, and society. Current studies have suggested that MSC-Exos can play an important role in

various aspects of wound healing and hold sufficient promise for promoting diabetic wound healing. However, recent clinical applications of MSC-Exos in DCW repair are still limited. Moreover, clinical translational issues, such as exosome production, isolation, purification, and storage processes, the most effective route of administration and dose, and efficacy evaluation remain. Accurate and efficient exosome products need to be established, and experiments in animals that have a greater resemblance to human skin tissues and clinical trials need to be initiated as soon as possible to validate the optimal dosage and administration, and efficacy evaluation for using MSC-Exos to provide safety assurance for further clinical applications. Modification of MSC-Exos and integration with biomaterials to improve their efficacy and reduce their elimination rate may be a promising direction. We look forward to the clinical application of MSC-Exos for diabetic wound healing.

FOOTNOTES

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MINIREVIEWS

Prehabilitation of overweight and obese patients with dysglycemia awaiting bariatric surgery: Predicting the success of obesity treatment

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Abstract

Bariatric surgery offers the best health results in overweight and obese patients but is not a risk and/or complication-free treatment. In cases with additional hyperglycemia, the burden of surgery can be even higher and alter both short-term and long-term outcomes. Although bariatric surgery offers glycemic improvements and in the case of early onset diabetes disease remission, weight loss results are lower than for obese patients without diabetes. Different multimodal programs, usually including interventions related to patients' performance, nutritional and psychological status as well as currently available pharmacotherapy before the surgery itself might considerably improve the immediate and late postoperative course. However, there are still no clear guidelines addressing the prehabilitation of obese patients with dysglycemia undergoing bariatric surgery and therefore no unique protocols to improve patients' health. In this minireview, we summarize the current knowledge on prehabilitation before bariatric surgery procedures in patients with obesity and dysglycemia.

Key Words: Bariatric surgery; Obesity; Dysgylcemia; Diabetes outcome; Prehabilitation

Core Tip: The prehabilitation of bariatric surgery patients is an insufficiently investigated area of research. Adequate perioperative preparation for patients awaiting bariatric surgery could present one of the main determinants of predicting the success of surgical treatment, especially in patients with associated dysglycemia. A combination of calorie restrictive diet, structured exercise program, psychological support, and anti-obesity pharmacotherapy should be implemented in the perioperative care of candidates for bariatric procedures. This multimodal approach has the most promising potential to promote 5% weight loss at least thus affecting chronic inflammation and insulin resistance, the main culprits of bariatric surgery resistance.

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INTRODUCTION

Obesity is a chronic debilitating disease with many health-related consequences. Nearly 39% of the worldwide adult population in 2019 met the criteria of being overweight and obese, and had multiple comorbidities[1,2]. In the case of additional derangements in glucose metabolism, such as glucose intolerance or diabetes whose incidence increases with increasing body mass index (BMI), patients have an even worse long-term prognosis, with accentuated cardiovascular risk, morbidity, and mortality[3].

Even the accumulation of free fat mass in the legs, arm, and trunk area is reversely associated with diabetes as was demonstrated in a recent study[4]. Moreover, when weight reduction results (due to lifestyle interventions, pharmacotherapy, or metabolic surgery) are compared to obese patients with and without diabetes, later are always more humble, suggesting the necessity for a structured and multimodal approach[5].

Weight management aimed at weight reduction has favorable metabolic, and mental health benefits in obese patients. A healthy lifestyle, including physical activity, is one of the pillars of weight management, impacting overall cardiometabolic health and well-being[6]. In addition, newly available anti-obesity drugs can lead to potent weight loss results, but the most powerful strategy includes bariatric surgery. Different surgical approaches can be selected, some with malabsorptive effects and others, such as gastric sleeve-resection do not have malabsorptive effects.

Malabsorptive procedures lead to nutritional risks, which might also exist preoperatively, regardless of patients' BMI. Therefore, preoperative nutritional status assessment and cardiorespiratory fitness status might be important parameters in decision making, treatment planning, and psychiatric evaluation. The Enhanced Recovery after Bariatric Surgery protocol suggests that a higher preoperative fitness level leads to improved outcomes and fewer postoperative complications[7]. Unfortunately, current medical care does not routinely include a physical exercise component for bariatric surgery patients. Moreover, < 10% of bariatric surgery patients meet the current physical activity recommendations, although it has been shown that two weeks before surgery, 40% of obese patients would feel ready to start exercise[8].

In addition, prehabilitation might be the key to improving responsiveness to metabolic surgery, especially in patients with dysglycemia, one of the common comorbidities in overweight/obese patients that must be addressed preoperatively[9].

In this minireview, we will focus on multimodal prehabilitation of patients undergoing bariatric surgery and specifically look into data on patients with coexisting dysglycemia.

ROLE OF EXERCISE

Exercise is a cornerstone of a healthy lifestyle and disease prevention, and sedentarism, lack of exercise, or nonattainment of physical exercise goals have been strongly correlated with chronic non-communicable diseases such as obesity, metabolic syndrome, and type 2 diabetes mellitus (T2DM)[10,11]. The inclusion of physical exercise in multimodal preconditioning programs for patients undergoing

different surgical procedures has been in the research scope of numerous investigations[12].

The role of exercise programs before and after bariatric surgery procedures might be important both from the aspect of reduction of perioperative and postoperative complications and as a means of retaining weight loss results achieved by surgery and acquisition of a healthy lifestyle[13,14]. Unfortunately, despite convincing beneficial outcomes reported from other surgical procedures, structured perioperative exercise programs are barely/rarely used perioperatively for bariatric procedures. According to the literature, physical exercise can contribute to approximately 4% excess weight loss, and when exercise is performed post-bariatric surgery, it results in an additional 3.6 kg weight loss[15]. The beneficial effects of exercise on anthropometric measures (weight loss, reduction of fat mass, and reduction of neck circumference) accompanied by improvement in physical performance (measured by the 6-min walk test) and quality of life are well documented[16,17]. There are, however, no clear recommendations on validated programs concerning starting the exercise before bariatric surgery, type of exercise, the intensity of exercises, duration of exercise sessions, or the comparison of different exercise types concerning short-term and long-term outcomes. Moreover, the literature is mainly focused on exercise performed post-bariatric surgery procedures and how it might help retain weight loss and cut cardiovascular risk compared to preoperative exercise programs[18,19].

A few studies that have assessed the value of preoperative exercise suggest benefits in fitness level and achievement of presurgery weight loss. Specifically, a 12-wk pre-bariatric surgery program including endurance and resistance exercises suggests improvements in fitness and quality of life-extending one year post-operatively[14,16,20]. In addition, studies using endurance and resistance training as a pre-bariatric surgery intervention reported improvements in weight and functional capacity, comorbidities, and quality of life[21,22].

Recently published data from a randomized controlled trial, although having major adherence issues, suggested the benefit of resistance exercises with elastic bands involving large muscle groups of the upper and lower extremities in the perioperative period of obese patients awaiting bariatric surgery together with respiratory prehabilitation[23].

Obese patients with dysglycemia (prediabetes or diabetes) are at higher risk of diabetes and obesity-related comorbidities[24].

In the study by Hickey *et al*[25] a seven-day 60-min daily exercise program led to a significant decrease in fasting plasma insulin level, suggesting improvements in tissue insulin sensitivity, which is particularly important for overweight/obese patients with dysglycemia. During 24 wk of low-intensity endurance training, in addition to anthropometric parameter measurements, Marcon *et al*[26] found substantial improvements in systolic and diastolic blood pressure, lipid and glucose levels, and patients' performance. A study by Woodlief *et al*[27] focusing on exercise dose after Roux-en-Y gastric bypass surgery showed that even a modest amount of structured exercise leads to improvements in insulin sensitivity but that higher volumes of exercise are needed for more profound health benefits.

On the other hand, Gilbertson *et al*[28] investigated the effects of aerobic exercise (30 min/d, 5 d/wk, at home, walking at the intensity of 65%-85% peak heart rate during 30 d) on metabolic and short-term postoperative outcomes of bariatric patients. They found a significant decrease in calorie intake, increase in VO_2peak, decrease in high sensitivity C-reactive protein (hsCRP), cytokeratin 18 and improvement in quality of life, decreased sugar intake, improved whole-body insulin sensitivity, and glucose levels together with a shorter hospital stay in patients who were in the exercise group[28]. Moreover, from the aspect of choosing a better exercise type, interval training might be superior to moderate-intensity continuous training in terms of reducing fat mass[29].

The main problem in objectively assessing the contribution of exercise programs on weight loss outcomes, besides the lack of randomized controlled trials, is the lack of structured exercise, poor patient adherence, and the self-reported measurement of exercise limiting interpretation of the results.

ROLE OF DIET

Restrictive calorie intake is widely advocated for obese patients undergoing metabolic surgery, and a weight loss of 5%-10% is generally mandatory before patients are considered as candidates for bariatric surgery, primarily as a means of assessing patient's motivation and adherence to follow-up after the surgery[30].

Currently, different dietary interventions mainly investigated in a non-randomized and uncontrolled manner, such as a low-calorie diet (800/1200 kcal daily) or a very-low-calorie diet (600 kcal per day), were shown to reduce weight preoperatively (4.2% and 5.8%, respectively) with no difference in inducing a reduction in liver volume and having similar effects on surgical complications, length of hospital stay and biochemical parameters[31]. In addition, very low-calorie ketogenic diets have recently been investigated in the context of weight reduction in obese patients. Although concern is raised due to their ability to induce catabolism, enhance oxidative stress response, and through high protein intake, induce a negative metabolic response, data available from a few non-randomized studies suggest that the mentioned dietary regimen when used 30 d before bariatric surgery and in a sequential way with low calorie and a very low-calorie diet adds beneficial effects in terms of better weight

reduction, waist circumference, visceral fat reduction, and improvement in glycemic and lipid profiles accompanied by a mean 30% reduction in liver volume[32-34].

It is still unclear whether overweight and obese patients benefit from short-term dietary weight loss interventions while changes in the level of circulating mediators of appetite such as leptin, ghrelin, and GIP might favor long-term weight regain[35]. Moreover, overweight/obese patients might also be at nutritive risk, which might escalate if restrictive diets are not controlled[36]. Numerous studies reported multiple micronutrient deficiencies in obese patients[37-39], while Schiavo *et al*[40] showed that preoperative micronutrient supplementation leads to the prevention of micronutrient deficit in the postoperative period. Therefore, current guidelines support the preoperative nutritional status screening of all patients awaiting bariatric surgery[41].

A meta-analysis including 6060 patients showed significant weight reduction achieved through preoperative dietary restriction led to significant weight loss and 27% shorter duration of hospital stay, but with no difference regarding perioperative morbidity and mortality[42]. Stefura *et al*[43] prospectively collected data from 909 bariatric patients treated by ERAS principles and depicted predictors of success in losing > 5% of initial weight as positive (diabetes mellitus, obstructive sleep apnea, and previous surgery) or negative (steatohepatitis, respiratory disorders). Although there was no influence of preoperative weight loss on perioperative morbidity or mortality, patients who lost > 5% in the perioperative period had better weight loss results post-surgery[43].

The efficacy of calorie restriction (very-low-calorie diet and more recently very low-calorie ketogenic diet) in weight loss potential is an interesting bridging therapy before bariatric surgery but is still under debate due to the lack of large randomized studies addressing the issues around the effect on postoperative complications.

ROLE OF PHARMACOTHERAPY IN PREHABILITATION

A certain number of individuals are resistant to the weight loss effects of bariatric surgery due to multiple reasons such as the level of chronic inflammation, presence of T2DM, age, gender, and ethnicity[44].

Chronic inflammation and increased circulating levels of pro-inflammatory cytokines such as interleukin-6 and tumor necrosis factor-α caused by white visceral adipose tissue could be one of the main reasons for bariatric surgery resistance independent of all other factors[45]. In responsive individuals, bariatric surgery reduces pro-inflammatory cytokines promoted by weight loss and attenuates insulin resistance[46-48]. Therefore, reducing pre-operative inflammation could improve response to bariatric surgery[49].

To date, several studies have demonstrated that severe dysglycemia, duration of diabetes, and anti-hyperglycemic therapy at the time of surgical procedure are the key factors in predicting response to bariatric surgery[50-54]. Whether hyperglycemia or insulin resistance are the main culprits in bariatric surgery resistance remains to be seen but improving glycemic regulation and insulin sensitivity could be the most important pre-operative pharmacological targets to improve responsiveness to bariatric surgery.

In addition, unchangeable factors, including aging, female sex[55,56], and Hispanic and African American races[57], are associated with higher rates of bariatric surgery failure. Therefore, influencing modifiable risk factors seems to be the most reasonable approach to improve the success of bariatric procedures.

Although lifestyle modifications such as physical activity and diet play a major role in the prehabilitation of bariatric patients, adherence to lifestyle changes remains an elusive and poorly attainable goal [42]. Implementing pharmacological options that reduce insulin resistance and chronic inflammation by lowering body weight preoperatively in patients with or without diabetes has great potential to improve the response to bariatric surgery.

There are several weight loss agents available on the market. One of the most frequently used is liraglutide, a long-acting glucagon-like peptide 1 receptor agonist (GLP 1 RA) approved for the treatment of T2DM and obesity due to its mechanism of action based on delayed gastric emptying, central reduction of appetite, and stimulation of glucose-dependent insulin secretion[58,59]. The efficacy and safety of liraglutide 3 mg daily were assessed in the phase III clinical trial program SCALE, demonstrating greater improvement compared to placebo with regard to HbA1c, blood pressure, lipid reduction, and health-related quality of life in overweight people and obese patients[58-61]. However, most research seems to focus on the role of liraglutide in post-operative management, preventing weight regain, and promoting further weight loss. At the same time, data on perioperative administration are scarce. The effectiveness of liraglutide in the prehabilitation of bariatric patients was demonstrated for the first time in a retrospective cohort analysis by Wood *et al*[62] in which therapy with GLP-1 receptor agonists in combination with other anti-diabetic medication prior to bariatric surgery led to higher T2DM remission rates, short- and long-term, compared to therapy with other anti-diabetic medications alone[62,63]. Recently, a case series also demonstrated the potential benefit of short-term therapy with liraglutide prior to bariatric surgery[64]; however, data from randomized

clinical trials (RCTs) are lacking.

Presently, there are several retrospective studies demonstrating the efficacy of liraglutide therapy in patients that underwent bariatric surgery with inadequate weight loss or weight regain[65,66], including one RCT investigating liraglutide effects compared to placebo on total weight loss and excess body weight loss added early after laparoscopic sleeve gastrectomy in obese individuals[63]. Liraglutide significantly improved the resolution of dysglycemia and weight loss effects of the surgical procedure compared to placebo.

Another promising agent from the same class is semaglutide, a long-acting GLP 1RA with proven effects on diabetes management and weight loss and recently approved by the FDA for both indications.

Semaglutide has improved pharmacokinetic properties compared to liraglutide, enabling once-weekly administration and greater efficacy[67]. In a phase III clinical trial assessing the efficacy and safety of semaglutide 2.4 mg in obesity treatment, greater reductions in body weight were observed after 68 wk with once-weekly semaglutide 2.4 mg sc *vs* placebo (mean change from baseline -14.9% *vs* -2.4%; ETD -12.4%; 95%CI: -13.4 to -11.5; $P < 0.001$)[68-71]. Similar results were found in a 68-wk phase III study (STEP 3) comparing the effects of semaglutide 2.4 mg *vs* placebo in overweight or obese adults without diabetes. The mean body weight decreased 16% with semaglutide, compared to 5.7% with placebo ($P = 0.0001$)[70]. No data are available on semaglutide in the prehabilitation of bariatric patients.

Tirzepatide belongs to an emerging new class of drugs called twincretins, dual receptor agonists of the glucose-dependent insulinotropic polypeptide (GIP) and GLP-1[72]. In the phase III clinical trial program SURPASS, designed to assess the efficacy and safety of tirzepatide 5, 10, and 15 mg as a treatment to improve glycemic control in patients with T2DM, tirzepatide demonstrated impressive results in terms of glycemic regulation and weight management[73,74]. In SURPASS-2, a higher dose of tirzepatide (15 mg) had more pronounced weight loss effects compared to semaglutide 1 mg (13.1% *vs* 6.7%) as well as better anti-hyperglycemic effects (2.3% *vs* 1.86%)[74].

Older anti-obesity medications such as orlistat, phentermine/topiramate, and naltrexone/bupropion have low efficacy and cause a drop in body weight up to 3%-7% compared to placebo with unfavorable safety profiles[75]. Liraglutide also induces similar weight loss but with a more acceptable safety profile. Consequently, the efficacy of semaglutide 2.4 mg and tirzepatide 15 mg in terms of weight loss effects is extremely significant, highlighted by the fact that approximately 75% of patients treated with semaglutide 2.4 mg or tirzepatide 15 mg experience 10% to 15% body weight loss accompanied by well-known side-effects such as nausea, vomiting, diarrhea and obstipation[76].

Therefore, these new agents could represent a new era in optimizing the medical care of bariatric surgery patients with the potential to significantly influence surgery outcomes. Further prospective randomized trials are necessary to determine the significance of these new classes of anti-obesity medications in the prehabilitation of bariatric surgery patients.

ROLE OF PSYCHOLOGICAL SUPPORT

Numerous studies have demonstrated a link between obesity and psychological disorders in patients awaiting bariatric surgery, the most common being anxiety, depression and binge eating disorders (BED)[77-79]. However, the effect of psychological status perioperatively on the success of bariatric surgery remains to be clarified due to large heterogeneity within the same psychiatric diagnosis influencing eating patterns. For instance, in a recently published study, better weight loss was associated with depression and BED diagnosis[80] as opposed to other findings linking higher levels of psychopathology with the diminished success of weight reduction[81,82]. Moreover, the results of the latest meta-analysis including published studies on psychological interventions in patients undergoing bariatric surgery were ambiguous regarding the usefulness of psychological support on bariatric surgery outcomes[83]. Therefore, further research on this topic is needed to assess if the benefit of psychological therapy really exists.

FUTURE IMPLICATIONS

Without a doubt, lifestyle modifications based on implementing structured exercise programs and nutritional plans offer great benefits in the prehabilitation of patients awaiting bariatric surgery, especially those with associated dysglycemia. The ultimate goal is achieving a minimum 5% weight loss and improving cardiorespiratory fitness and increasing basal rate consequently promoting further postoperative weight loss and bariatric surgery responsiveness as well as reducing postoperative complications and mortality. However, clear recommendations regarding the most efficient exercise protocols and calorie-restrictive diets are lacking and further prospective studies are needed to establish effective and safe protocols to upgrade peri and postoperative care as well as the short- and long-term outcomes of surgery. One should not forget the influence of patient characteristics, psychological profile, social conditions, and behavioral responses to the operation, which also have a great impact on surgery success requiring the development of protocols for psychological support. Furthermore, current

Table 1 Proposed recommendations for the perioperative care of all bariatric surgery patients, especially those with associated dysglycemia

Prehabilitation- treatment modality	Potential advantages and clinical rationale
Exercise	
Resistance and endurance training	Short- and long-term improvements in weight and functional capacity, comorbidities, quality of life, improvements in tissue insulin sensitivity
Aerobic training	Short-term decrease in calorie intake, improvement in quality of life, improved whole-body insulin sensitivity, decrease in glucose levels, shorter hospital stay
Nutritional interventions	
Low and very low calorie and ketogenic diet	Better weight reduction, visceral fat reduction, improvement in glycemic and lipid profiles, mean 30% reduction in liver volume
Pharmacotherapy	
GLP 1 receptor agonists	Higher T2DM remission rates, better body weight reduction, improvement in glycemic and lipid profiles
Psychological support	
Preoperative counseling and education	Reduced anxiety, depression, and fear, positive influence on eating disorders

GLP 1: Glucagon-like peptide 1; T2DM: Type 2 diabetes mellitus.

anti-obesity pharmacotherapy such as GLP-1 RA and in the future twincretins offers a significant opportunity to improve the peri and post-operative care of bariatric patients, acting in synergy with exercise and calorie-restrictive diets. Moreover, the degree of obesity and age influence the choice of treatment strategy or protocol in perioperative care. However, there are significant shortcomings as most of the research to date has been focused on the postoperative care of bariatric surgery patients, while research on perioperative care has been somewhat neglected.

CONCLUSION

We have attempted to summarize current knowledge and propose recommendations for perioperative care of all bariatric surgery patients, but with special emphasis on those with disturbances of glucose metabolism (Table 1). Future studies should focus on the development of perioperative treatment protocols consisting of the most optimal combination of lifestyle changes and pharmacotherapy thus optimizing response to bariatric surgery, ultimately improving both short -and long-term outcomes by reducing the incidence of T2DM and cardiovascular disease.

FOOTNOTES

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MINIREVIEWS

Diabetic foot ulcers: A devastating complication of diabetes mellitus continues non-stop in spite of new medical treatment modalities

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Abstract

Diabetic foot ulcer is a devastating complication of diabetes mellitus and significant cause of mortality and morbidity all over the world and can be complex and costly. The development of foot ulcer in a diabetic patient has been estimated to be 19%-34% through their lifetime. The pathophysiology of diabetic foot ulcer consist of neuropathy, trauma and, in many patients, additional peripheral arterial disease. In particular, diabetic neuropathy leads to foot deformity, callus formation, and insensitivity to trauma or pressure. The standard algorithms in diabetic foot ulcer management include assessing the ulcer grade classification, surgical debridement, dressing to facilitate wound healing, off-loading, vascular assessment (status and presence of a chance for interventional vascular correction), and infection and glycemic control. Although especially surgical procedures are sometimes inevitable, they are poor predictive factors for the prognosis of diabetic foot ulcer. Different novel treatment modalities such as nonsurgical debridement agents, oxygen therapies, and negative pressure wound therapy, topical drugs, cellular bioproducts, human growth factors, energy-based therapies, and systematic therapies have been available for patients with diabetic foot ulcer. However, it is uncertain whether they are effective in terms of promoting wound healing related with a limited number of randomized controlled trials. This review aims at evaluating diabetic foot ulcer with regard to all aspects. We will also focus on conventional and novel adjunctive therapy in diabetic foot management.

Key Words: Diabetic foot ulcer; Peripheric artery disease; Macrovascular complications; Neuropathy; Wagner classification; Intralesionar growth factor treatment

Core Tip: Diabetic foot ulcers (DFU) are one of the most common problems and devastating complications of diabetes, and affect 15% of all diabetic patients and result in significant morbidity, mortality, and financial burdens. The management of DFU is usually complex and challenging to clinicians in clinical practice. This review article aims at summarizing the etiopathogenesis and classification of DFU. It also highlights novel adjunctive treatment modalities as well as conventional management for DFU.

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INTRODUCTION

Diabetic foot ulcers (DFU) are common clinical problems and devastating complications of diabetes, and affect 15% of all diabetic patients and results in significant morbidity, mortality, and financial burdens [1]. Five-year risk of mortality for a patient with diabetic foot ulcer is 2.5 times higher than the risk for a patient without[2]. Approximately 20% of moderate or severe DFU could cause some level of amputation. Moreover, 74% of them also have a risk of renal replacement therapy at 2 years[3]. This high mortality rate is also related with coexisting comorbidities such as cardiovascular or cerebrovascular diseases.

The pathophysiology of DFU is based on a triad of neuropathy, peripheral arterial disease, and concomitant secondary bacterial infection. Peripheral neuropathy could lead to intrinsic muscle atrophy and functional anatomical changes in the foot[4]. Eventually, progressive secondary foot infection penetrating deep facia, tendons, and joints could develop with repetitive inattention trauma. Infection could play a significant role for half of major lower limb extremity amputations. Recent studies indicate some risk factors for the development of DFU. These are as follows: Longer than 10 years of duration of diabetes, male gender, older patients, presence of comorbidities including nephropathy, neuropathy, and peripheral vascular disease, and history of foot ulceration[4-6].

The management of DFU is usually complex and challenging to clinicians in clinical practice. Costs of diabetic foot ulcerations have been increased to the treatment cost of many common cancers. Estimated costs of DFU management are greater than 1 billion in both developed and developing countries. Therefore, foot ulcers should be treated immediately by a multidisciplinary expert team for optimal outcomes. The treatment of DFU requires an immediate decision and systematic approach that comprises of maintaining arterial blood flow, treating the infection appropriately, and removing the pressure from the wound[7]. In addition, several adjuvant therapies are becoming a popular form of diabetic foot treatment. During the past 10 years, there has been an increasing amount of novel, basic science-based approaches and developments for adjuvant therapies including wound dressing, hyperbaric oxygen therapy, or growth factor formulations for efficient local delivery[8-10]. None of them had definitive results that improved wound healing and these approaches still need clinical validation.

The review highlights novel adjunctive treatment modalities as well as conventional management for DFU.

PATHOPHYSIOLOGY

DFU is a serious result of risk factors including neuropathy, peripheral arterial disease, and secondary infection. Peripheral neuropathy could play a major role by producing intrinsic muscle atrophy and consequently leading to biomechanical anatomical changes on the feet such as hammer-toe formation, pes-planus, and pes-cavus, which lead to high-pressure zones of the foot[11,12] (Figure 1).

Diabetic neuropathy and foot ulcer

Diabetic neuropathy has multiple manifestations within the diabetic foot because it comprises sensory, motor, and autonomic fibers. The majority of patients with diabetes (66%) face peripheral neuropathy in the lower extremities[12]. Especially distal sensory neuropathy, the most type seen of all diabetic neuropathies, is a major risk factor for DFU but it is extremely variable, as it ranges from severely painful symptoms to a completely painless variety that may present with an insensitive foot ulcer[13]. Diabetic peripheral neuropathy mainly affects lower legs and the feet as a stocking-glowing distribution and it could cause the loss of the Achilles reflex which can be the first sign of these changes. The anatomy of the foot arch could change with the atrophy of the lumbricals and interosseus muscles and a

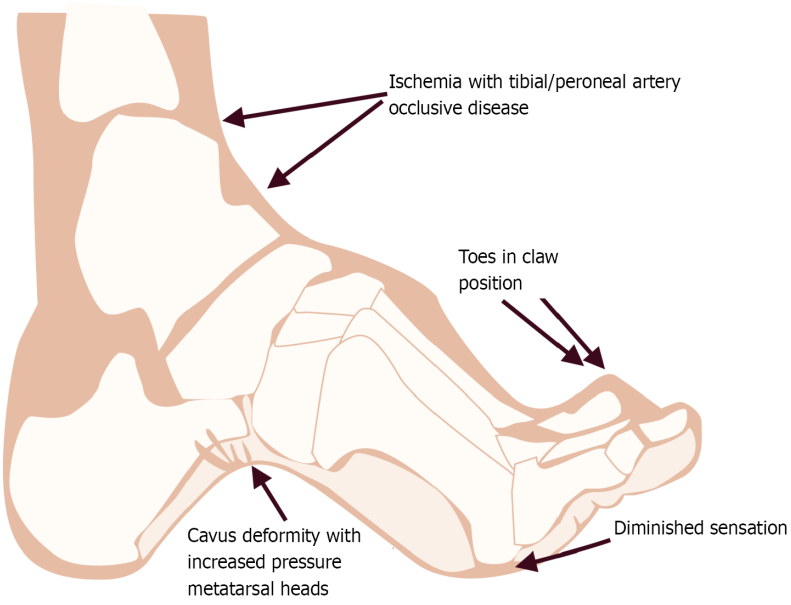

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Figure 1 Ethiopathogenesis of diabetic foot leading to vascular complications.

relative increase in the extensor tendon forces called "claw" deformity in the toes[14]. On the other hand, the onset of sensory neuropathy can manifest as loss of proprioception, pressure sensation, vibratory perception, and impaired gait[15]. Sensory neuropathy usually progresses gradually with the insidious appearance of symptoms that may be intermittent in the early stage. C-type fiber, which is a responsible for the sensorial transmission, results in inappropriate reaction to a painful stimulus[16]. Ulceration and infection could occur with repetitive trauma, and decreased sensation and proprioception could also predispose skin injury by producing atrophy and dislocation of protective plantar fat pads. Moreover sudomotor dysfunction could develop with diabetic autonomous neuropathy and it is also associated with foot ulceration due to dry skin and itching[17]. Several methods are developed to evaluate the sudomotor function. They are thermoregulatory sweat testing, quantitative sudomotor axon reflex, quantitative direct and indirect reflex test, and indicator plaster[18-20]. Among of them, indicator plaster represents a rapid, simple method based on color change from blue to pink at the plantar foot region.

Since defining neuropathic symptoms could be difficult due to fluctuation of the symptoms, a diagnosis of neuropathy may be difficult. Small fiber function can be determined by pinprick and a cold or warm thermal stimulus to the distal sensation. The proximal sensory abnormality could be determined by moving the individual test paradigms proximally. Positive stimulus (allodynia and hypersensitivity) for diabetic neuropathy, can be showed by measuring the intensity or area of these phenomena. Negative symptoms in diabetic neuropathy are also numbness, no sensation, poor balance, or muscle weakness. Clinical examination in these patients can include quantitative sensory testing (QST) to asses this sensory stimulus and also provide the advantage of directly assessing the degree of sensory loss in the foot. Some of the more commonly used techniques are "Semmes-Weinstein monofilaments", ''thermal and cooling threshold'', "perception of vibration", and "computer-assisted sensory examination"[21-23].

Semmes-Weinstein monofilament includes nylon filaments which have variable diameters and is one of the clinical tests that measure the response to a touching sensation of the monofilaments using a numerical quality. Inability to perceive pressure of 10 g (5.07) by the monofilament has been shown in subjects who are at risk for neuropathic foot ulceration (Figure 2). This is a very easy and applicable examination in busy outpatient clinics and reveals diabetic foot ulcer risk. It is recommended to all practitioners[24].

Perception of vibration, which is called deep sensation impairment, is usually one of the earliest signs of peripheral diabetic neuropathy. Tuning forks (128 Hz) are generally used in general practice; these determine the perception of vibration through the application on distal bony prominence of the great toe bilaterally and other bony prominences such as the medial malleolus. Sensitivity of tuning fork is approximately 53% and it is less predictive for the development of foot ulceration compared to using the monofilament test[25].

Although electrophysiology is not required for clinical diabetic neuropathy evaluation, it quantitatively assesses large fiber involvement in diabetic neuropathy. While these QSTs are convenient techniques in daily clinical practice, simple clinical instruments including Michigan neuropathy screening instrument and simplified neuropathy disability score can be used to assess neurologic

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Figure 2 Monofilament test is a diagnostic tool to detect diabetic peripheral neuropathy. When the nylon line bends, the force is 10 grams. It is used for diabetic foot contact and stress testing.

deficits. Now these scoring systems become using in a number of ongoing trials with new therapies for diabetic neuropathy[26,27].

Although it is not the main topic of this review, briefly neuropathy management is done symptomatically and current therapeutic agents which are used, including tricyclic antidepressants, serotonin and noradrenalin reuptake inhibitors, and anticonvulsants, have efficacy in diabetic neuropathy[28]. Among these new anticonvulsants, pregabalin and gabapentin have been shown to be more convenient in the treatment of painful syndromes in recent articles[29].

Callus deformity and plantar shear stress

Calluses have been defined as hyperkeratosis caused by excessive mechanical loading. Calluses increase pressure of plantar area and the risk of DFU[30]. Significant risk factors for callus deformity in patients with diabetic neuropathy are foot deformity, limited joint mobility, repetitive stress of walking, and ill-fitting shoes[31]. Calluses are frequently developed under bony prominence including the metatarsal head. Proprioceptive loss due to sensory neuropathy and metatarsal heads leads to increased pressure and load under the diabetic foot. It has been reported that callus deformity may be related with a relative risk of 11 for ulcer development (Figure 3). As a result, removal of plantar callus is associated with reduced plantar pressure and thereby reduced foot ulcer risk.

Charcot neuroarthropathy

Charcot neuroarthropathy (CN) has been determined to be a chronic and destructive disease of the bone structure and joints in patients with neuropathy. The precise incidence of CN in persons has previously been estimated to be between 0.1%-0.4%[31]. Etiopathogenesis of CN is complex and based on varied degrees of neuropathy. Typical clinical symptom is characterized by painful or painless bone and join destruction in limbs that have lost sensory innervation. Although the clinical management of CN has many clinical challenges, it is generally characterized with asymptomatic nature such as ankle sprain, cellulitis, and thrombosis[32]. A diagnosis of CN is based on primarily on thorough history and physical examination, with corroborating laboratory investigations and diagnostic imaging. Modified eichenholtz classification is commonly used for description in the clinical stage[33] (Figure 4). According to this classification, stage 0 is mild inflammation, soft tissue edema, and normal X-ray, stage 1 is severe inflammation and abnormal X rays with microfractures, stage 2 is coalescence and end of bone resorption, and stage 3 is definitive bone remodeling with chronic CN.

Initial weight-bearing radiography can show demineralization, fragmentation, joint dislocation, osseous debris, and joint space obliteration[34]. But routine radiography gives limited information about the differentiation of CN from osteomyelitis. Imaging techniques including magnetic resonance imaging (MRI) or PET/CT scans highlight the inadequacies of clinical examination and radiographs in assessing the CN stage[35]. Orthopedic surgery is often required to correct severe foot deformities when conservative measures including physiotherapy are not effective (Figure 5).

Peripheral vascular disease

Peripheral vascular disease is characterized as a chronic arterial occlusive disease of the lower extremities and varies in severity. Patients with peripheral arterial disease have usually intermittent claudication, rest pain, and tissue loss with or without gangrene[36]. Rest pain is shown in these patients and related with chronic sensory nerve ischemia. Resting pain emerges as a diffuse burning or

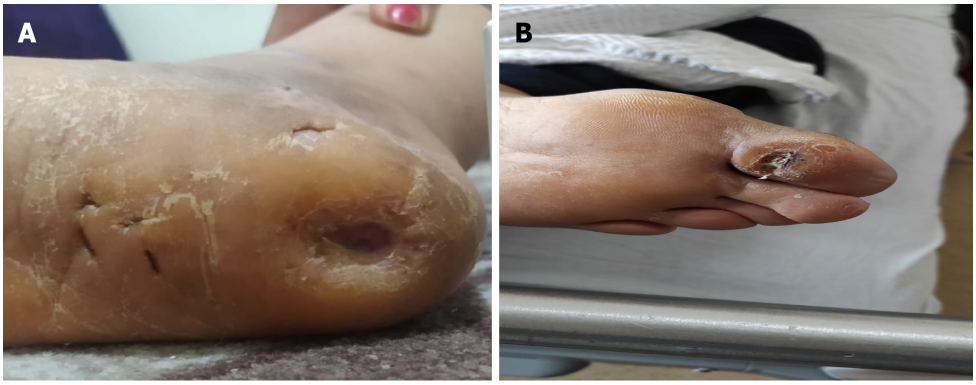

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Figure 3 Callus formation as a presentation of diabetic neuropathy.

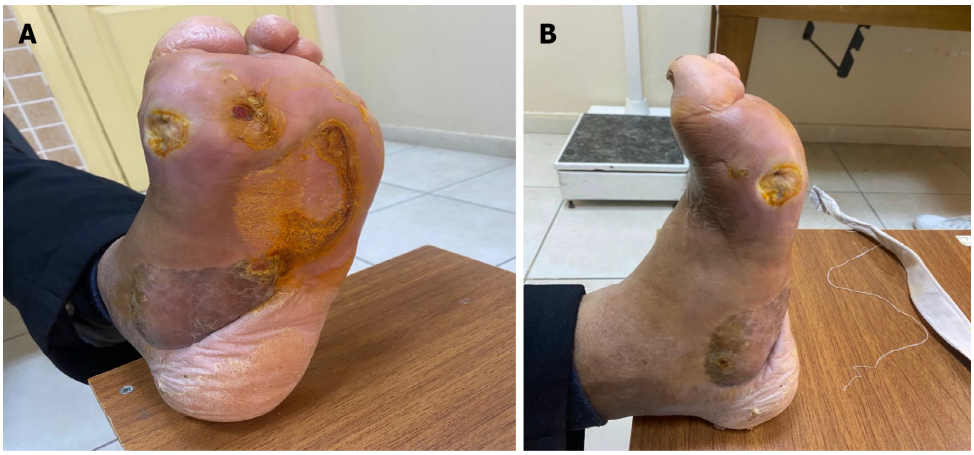

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Figure 4 Charcot neuroarthropathy is a chronic devastating and destructive disease of bone structure and joint in patients with neuropathy. A and B: Rocker-bottom foot deformity to charcot process.

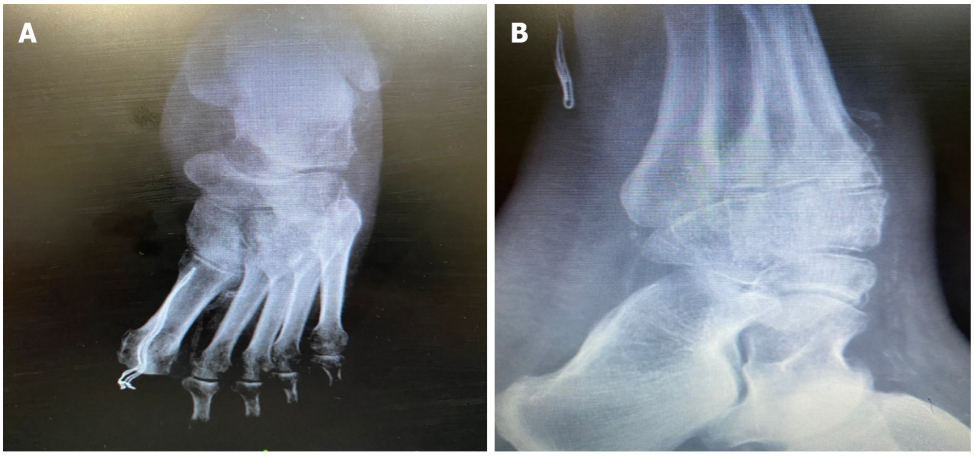

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Figure 5 Radiographic findings. A and B: Radiographic findings of charcot neuroarthropathy.

aching pain in the fore foot[37]. Intermittent claudication and rest pain are the clues to diagnosis, though it can lack symptoms or can be difficult to be attributed exclusively to peripheral vascular disease. Any worsening of walking quality or speed should be taken into account, as well as fatigue, pain, cramps, discomfort, or burns in the buttocks, thighs, or feet. The extent of ischemia and symptoms are related with location vascular lesion as well as the development of collateral circulation[38].

Generally, patients with aorto-iliac disease have buttock and thigh pain, and femoral disease causes calf discomfort. Tibio-peroneal disease generally does not have claudication, although some patients will complain of foot pain or numbness while walking.

Pulse palpation (distal pedis, posterior tibial, posterior tibial, popliteal, and femoral arteries), which can be a simple, cheap, and comfortable clinical examination, should be performed on all diabetic patients during the follow-up examination. Ankle-brachial index (ABI) has emerged as a relatively non-invasive tool for the diagnosis of peripheral arterial disease[39]. The measurement of ABI (ratio of systolic blood pressure on the ankle to the systolic blood pressure in the upper arm) is normal in the 1.0-1.4 range, obviously pathologic under 0.9. An ABI over than 1.4 is also considered as abnormal, reflecting calcified and stiffed arteries. Doppler ultrasound examination and computed tomography angiography are often used as non-invasive tests[40]. Intra-arterial digital subtraction angiography is defined as the gold standard for arterial imaging because of its high spatial resolution. It has the advantage of allowing endovascular therapy during the same procedure and it is also extremely accurate for the smaller vessels of the ankle and foot. But it has a disadvantage for patients with renal insufficiency [glomerular filtration rate (GFR) < 35 mL/min/1.73 m^2][37].

Infection and osteomyelitis

Infection in ulcerated diabetic foot is a primary cause of morbidity and mortality. It is well known that diabetic patients are prone to infectious diseases because of the diminished host defenses, including inadequacy in leukocyte capacity and morphologic alterations to macrophages, elevation of proinflammatory cytokines, and impairment of diabetic polymorph-nuclear cell functions (chemotaxis, phagocytosis, and killing)[41,42]. Many organisms can cause diabetic foot infection, but Gram positive cocci, especially *staphylococci* (*S. aureus*), are the most common cause pathogens[43]. Peripheral neuropathy, angiopathy, and a lack of attention to foot hygiene such as using poorly fitting footwear are the major factors in the development of infection. Abrasions, rashes, and loss of skin integrity due to fungal infection can be facilitating factors for diabetic foot infection. Approximately 60% of foot infections starts in webbed spaces and 30% in nails, while 10% are secondary punctures[44]. Ulcers > 60 mm^2 in size, purulent discharge from sinus tract, presence of sausage toe, and erythrocyte sedimentation rate > 70 mm/h suggest the presence of underlying osteomyelitis[45]. Osteomyelitis could be able to occur after the spread of superficial infection of the soft tissue on the adjacent bone or marrow. Although numerous expensive radiology techniques are available to diagnose osteomyelitis, specific clinical signs of inflammation (swelling, erythema, warmth, tenderness, pain, or induration) and the use of simple metal probes can often be used to make clinical diagnoses. In initial clinical visit, plain radiographs should be obtained to determine the extent of osseous erosion, as well as to assess anatomy for surgical planning. Further scanning tests including MRI and bone scans could be performed for patients with neuropathic osteoarthropathy or multifocal disease[46-48].

CLASSIFICATIONS OF FOOT ULCERS

Identification and classification of patients with DFU should be performed to see whether hospitalization, intravenous (IV) broad spectrum antibiotics, or surgical consultations will be required or not. An accurate defining of ulcer characteristics such as size, depth, appearance, and location allows for the mapping of progress during management of DFU. There have been several classification systems that have been broadly externally validated for ulcer healing and lower extremity amputation, and they are Meggitt-Wagner, University of Texas, Infectious Disease Society of America (IDSA), perfusion, extent, depth, ischemia, sensation (PEDIS), SINBAD, and Wound, Ischemia and foot Infection (WIfI) classification[49-51].

The Meggitt-Wagner classification was the first announced classification system; however, it is not well validated and does not distinguish well between ulcer types for the main purpose of classification. This system consists of six different groups: 0, intact skin; 1, superficial ulcer; 2, ulcer reaching to tendons, joints, bones; 3, deep ulcer with abscess and osteomyelitis; 4, local gangrene; and 5, gangrene of the entire foot. It presents vascular perfusion only when gangrenous changes appear and infection when osteomyelitis is present (Table 1).

The University of Texas classification is well validated but it does not indicate neuropathy or depth of the ulcer area, which is considered to be one of the main determinants of the ulcer healing (Table 2). IDSA was reported as a guideline and diabetic foot is subclassified into the categories of uninfected, mild (restrictive involvement of only skin and subcutaneous tissue), moderate (more extensive), and severe (systemic signs of infection).

The PEDIS classification system is based on five features of the wound and it helps clinicians assess risk or prognosis for a person with diabetes and active foot ulcer. In addition, it was used for a clinical audit study in 14 European countries[52].

The SINBAD classification system matches each composing variable such as area, depth, infection, and neuropathy to a score (ranging from 0-6). This classification system has some benefits such as simple, quick to use and not requiring specialist equipment[53].

Table 1 Wagner-Meggit classification

Grade	Lesion
0	No open lesion
1	Superficial ulcer
2	Deep ulcer to tendon or joint capsule
3	Deep ulcer with abscess, osteomyelitis or joint sepsis
4	Local gangrene - fore foot or heel
5	Gangrene of entire foot

Table 2 University of Texas Classification system

	0	1	2	3
A	No open lesion	Superficial wound	Affected tendon/capsules	Affected bone/joint
B	With infection	With infection	With infection	With infection
C	Ischemic	Ischemic	Ischemic	Ischemic
D	Infection/ischemia	Infection/ischemia	Infection/ischemia	Infection/ischemia

The WIfI classification includes three prognostic factors that affect clinical management. These factors indicate wounds that are graded from 0 to 3, ischemia graded based on toe pressure index, and infection which is based on IDSA classification. The WIfI threatened limb classification has been shown to correlate well with a risk of major amputation but it is not enough make acute decisions about the treatment only by itself due to more confounding factors (Table 3).

Up to date there has been several classification systems but which classification to use is still controversial. Physicians who treat patients with DFU are concerned about which classification is recommended. They are still discussing the usefulness of these classifications, and the effects of such classifications on diagnosis or treatment remain unknown. Although there are some limitations in case of prognostic accuracy of the Meggitt-Wagner classification, this classification remains the most commonly utilized system in health care today.

MANAGEMENT OF DIABETIC FOOT ULCER

The main principle of management of DFU is to evaluate wound appearance (extent, size, depth, presence of infection, and wound duration) in detail. Clinicians should inspect the extent of tissue destruction and possible bone and joint involvement. After evaluating wound appearance, another major decision is whether the patient can be initially treated as an outpatient or needs hospitalization. Early superficial ulcer (< 2 cm) without systemic toxicity may be treated at home. If the patient has a deep gangrenous ulcer with infected or systemic symptoms or needs surgical treatment, hospitalization is advised.

Since diabetic ulcer healing depends on multiple factors, it should be evaluated by a multidisciplinary expert team. The treatment includes conservative and surgical interventions and there are some fundamental steps of diabetic foot management such as surgical debridement, dressing, wound offloading, vascular assessment, control of infection, glycemic control, and adjuvant therapies[54-57] (Table 4).

Surgical debridement

Debridement is a principal treatment of local wound healing and it involves removing hyperkeratotic epidermis (callus), necrotic dermal tissue, foreign debris, and bacterial elements from a wound bed. Debridement includes numerous forms such as mechanical, autolytic, enzymatic, and sharp[58,59]. Sharp debridement is more common to use, and it includes two forms, namely, clinic based debridement and surgery based excisional debridement. A combination of debridement methods could help to remove devitalized tissue that provides a nidus for bacterial proliferation and acts as a physical barrier for antibiotics. Debridement is the most important step of the wound healing. If necessary, it should be performed in every clinic visit by clinicians.

Table 3 Wound, Ischemia, and foot Infection classification

	Wound	Ischemia; toe pressure/*tcpo*$_2$	Infection
0	No ulcer and no gangrene	> 60 mm/Hg	Non-infected
1	Small ulcer and no gangrene	40-59 mm/Hg	Mild (< 2 cm sellulitis)
2	Deep ulcer and gangrene limited to toes	30-39 mm/Hg	Moderate (> 2 cm sellulitis)
3	Extensive ulcer or extensive gangrene	< 30 mm/Hg	Severe (systemic response/sepsis)

Table 4 Standard care of diabetic foot ulcer

Treatment	Description	
Debridement	Surgical debridement	Necrotic or non-viable tissue should be removed, regular (weekly) debridement is associated with rapid healing of ulcers
Dressing	Films, foams, hydrocolloids, hydrogel	Proper using of dressing materials could facilitate moist environment
Wound off-loading	Rock or bottom outsoles, custom-made insoles, some shoe inserts	Plantar shear stress should be removed
Vascular assessment	PTA or endovascular recanalization followed by PTA or by-pass grafting	Arterial insufficiency should be treated for improving wound healing
Control of infection	Appropriate antibiotic therapy according to pathogens	Deep tissue cultures should be obtained before antibiotic therapy, for mild infection treatment duration could be 1-2 wk but for moderate to severe infection, it should be 3-4 wk
Glycemic control		For better glycemic control, insulin treatment has been preferred in hospitalized patients with diabetic foot ulcers

PTA: Percutaneous transluminal angioplasty.

Dressings

After the adequate debridement period, soft tissue defect requires dressing materials for closure and/or coverage of wound area. Dressing with adequate biomaterials could provide wound healing processes and protect from contamination. Naturel skin is considered perfect wound dressing and therefore ideal wound dressing should try to mimic its properties. Since recent studies highlighted the role of wound environment, dressing also should be biocompatible and not provoke any allergic or immune response reaction and should be easily removed[60,61]. Alginate and collagen-alginate products, carboxymethyl-cellulose dressings, topical phenytoin, and hydrogels are types of dressings which are available[62,63]. But there have been still some questions to support the choice of any dressings or to promote healing of ulcer.

Wound off-loading

Plantar shear stress and vertical plantar pressure are major causative factor in the development and poor healing of DFU. Removal of pressure and/or redistribution of an increased weight bearing area of the foot can be achieved through off-loading strategies. Total contact casts and removable walkers are used for off-loading the diabetic foot[64]. Various therapeutic off-loading devices such as rock or bottom outsoles, custom-made insoles, and some shoe inserts (*e.g.*, metatarsal pads and medial arch supports) may reduce fore foot peak pressure[65,66]. Recently, The International Working Group[67] on the diabetic foot suggests the following recommendations: (1) Removal of pressure on ulcers is one of the main part of the treatment plan; (2) non-removable walkers are the preferred treatment; and (3) forefoot off-loading shoes or cast shoes may be used when above-the-ankle devices are contraindicated.

Vascular assessment

Revascularization of critically ischemic legs results in increased area perfusion after the procedure, which is in turn associated with a further reduced amputation rate. Arterial revascularization can be performed through open procedures such as a bypass or, in many cases, endovascular recanalization followed by PTA (percutaneous transluminal angioplasty) with or without adjunctive stenting[68]. Overall, the aim of vascular reconstruction is to restore direct pulsatile flow in at least one or more arterial structures, preferably feeding the wound.

Control of infection

The diagnosis of infection is based on parameters of inflammation and should always be classified

according to a preferred classification method. Antibiotic therapy is based on possible pathogens, presence of vascular disease, and the extent of foot infection. Hospitalization with parenteral antibiotic treatment is recommended when the infection penetrates to the deep fascia. Patients with chronic ulcer, prior antibiotic treatment, and recurring infection should be assumed to have methicillin-resistant *Staphylococcus Aureus* infection[69]. The spectrum can be broadened to cover Gram-negative aerobes in chronic infections. If a patient has a superficial ulcer without infection, empiric antibiotic treatment therapy is not recommended[70]. Oral therapy including trimethoprim/sulfamethoxazole or amoxicilline/clavulanic acid plus linezolid is recommended to patients with a superficial ulcer and presence of pedal pulses. Parenteral therapy such as vancomycin or daptomycin plus piperacillin/tazobactam or imipenem cilastatin or meropenem has been recommended to patients with systemic inflammation or ulcer/gangrene with penetration of deep fascia[71,72]. In addition, in order to avoid antibacterial resistance or other adverse outcome of therapy, it is the best approach to be followed.

Glycemic control

In hospitalized patients, intensive insulin treatment should be administered to patients with foot ulcer for better glycemic control. The evidence of glycemic control can accelerate the healing of foot ulcers and reduce the incidence of ulceration and amputation. Studies have shown that increased blood glucose level is correlated with decreased neutrophil function and suppression of inflammatory response[73-75]. Although there are limited randomized control trials (RCTs) which determine whether glycemic control improves wound healing better, in a meta-analysis which included 10897 patients without known history of peripheral arterial disease, it has been reported that intensive glycemic control was associated with a statistically significant decrease in risk of amputation of diabetic foot[76].

Adjuvant therapies

New treatment modalities have been developed since 2002. There are advanced wound therapy methods. In addition to conventional therapy, several types of treatment modalities are available, such as negative pressure wound therapy, which is also known as vacuum assisted closure (VAC), synthetic skin grafts, non-surgical debridement agents, topical growth factors, electrical stimulation, and hyperbaric oxygen chambers[77-83] (Table 5). Each has its own merits but economic constraints and patient compliance should be kept in mind.

Negative pressure wound therapy

Pressure wound therapy (VAC) was first declared and used in clinical practice by the German physician Fleischmann in 1993[77]. It has remarkable effect on wound drainage, also enhancing perfusion. VAC could be used for acute and chronic DFU and it promotes the growth of granulation tissue (Figure 6). It can be helpful in the postoperative management of DFU. According to the Wound Healing Society and European Wound Management Association[78]: (1) Wound infection should be well controlled after the debridement; (2) the risk of bleeding is well controlled; there is no active bleeding or exposed vascular damage on the wound or no risk of coagulation dysfunction; and (3) the risk of ischemia is treated and well controlled (ABI ranges from 0.9 to 1.3), and VAC therapy is recommended with class 1 evidence. After 1-2 rounds of VAC therapy, a comprehensive evaluation should be performed for an effective evaluation. It is recommended to continue VAC if there is growth of new granulation tissue on the wound surface with surrounding epithelization. If infection is aggravated, it should be stopped immediately. In recent years, VAC treatment has been used extensively for DFU management and studies have shown that it has certain advantages in preventing and controlling wound infections.

Synthetic skin grafts

Skin substitutes are classified into three groups based on the plasticity of preparation procedure and composition of the substitutes. Class 1 skin layer includes cultured epidermal equivalents which are formed of single-layered materials; Class 2 layer includes dermal components from processed skin or fabricated matrix protein; Class 3 layer also consists of dermal and epidermal components and skin grafts (allograft and xenograft)[80]. Class 3 layer is more popular and common to use. Although there are limited studies, wound healing can be promoted by using these agents.

Non-surgical debridement agents

Although sharp debridement can play a major role in wound healing, various techniques such as autolytic debridement with hydrogels, enzymatic debridement, maggot and larval debridement, and hydrotherapy are available[81,82]. But recent studies did not provide sufficient evidence to use one approach over other methods.

Hyperbaric oxygen chamber

Administration of 100% O_2 has some beneficial effects on wound healing. It not only causes increased blood and oxygen content in hypoxic tissue, but it also has antimicrobial activity due to enhanced mobility and bacteriophagic activity of leukocytes[83]. Studies show that hyperbaric oxygen stimulates

Table 5 Additional adjuvant care of diabetic foot ulcer

Item	Description
Negative pressure wound therapy (VAC)	Widely used, removal of the excess third space fluid from the area, reduction of bacterial load, increased granulation tissue, but RCTs have high risk of bias
Synthetic skin grafts (Bio-engineered skin substitutes)	Contribute to the new dermal tissue but limited data to prove benefit of these products
Non-surgical debridement agents (enzymatic debridement, autolytic debridement, hydroterapy, Maggot therapy)	Promoting fibroblast migration and improving skin perfusion but due to small RCTs, it has clinical bias for beneficial effect
Topical growth factors (EGF, VEGF, PDGF, FGF)	Promote healing non-infected foot ulcer and stimulating angiogenesis but limited trials confirming positive outcomes
Electrical stimulation	Bacteriostatic and bactericidal effect on foot ulcer but lack of evidence due to limited clinical trials
HBOC	HBOC therapy increases blood and oxygen content in hypoxic tissues and has antimicrobial activity, but it is unclear whether it has benefit in long term wound healing

RCT: Randomised controlled studies; HBOC: Hyperbaric oxygen chambers; VAC: Vacuum assisted closure; EGF: Epidermal growth factor; VEGF: Vascular endotelial growth factor; PDGF: Platelet derived growth factor; FGF: Fibroblast growth factor.

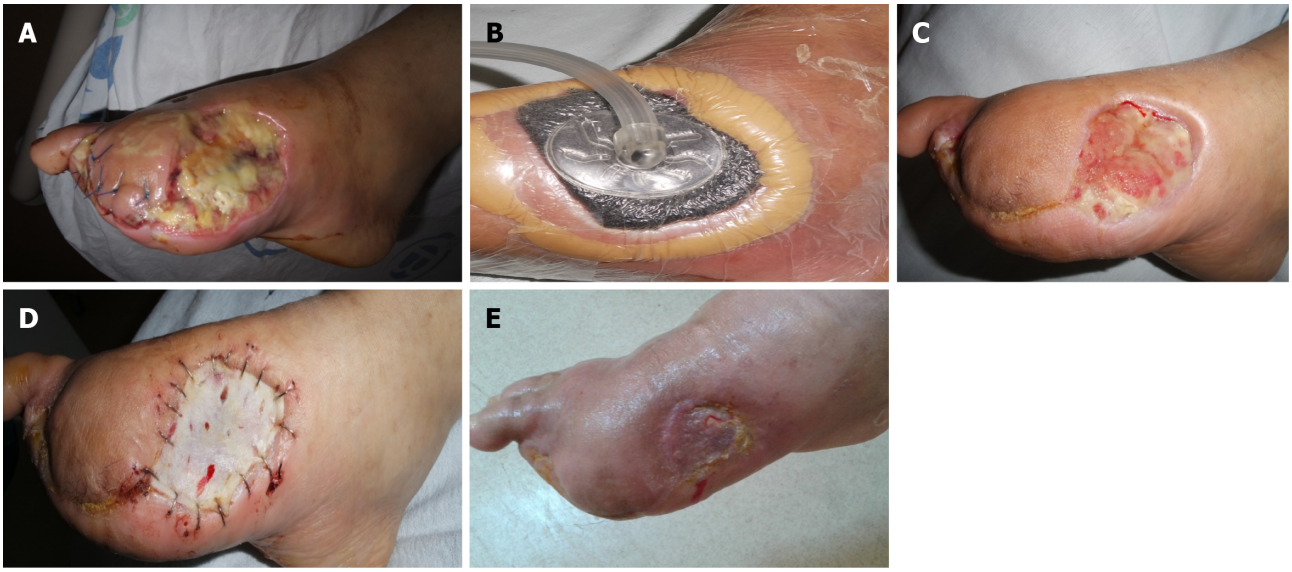

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Figure 6 Negative pressure wound therapy is considered as a better alternative therapy for the management of diabetic foot ulcer. A-E: This patient was treated with negative pressure wound therapy therapy after surgical therapy.

angiogenesis and increases fibroblast proliferation and collagen production. Some authors suggested that there are no definite results which display an improvement in DFU. There is large uncertainty associated with the evaluation of the cost-effectiveness of hyperbaric oxygen therapies. Up to date, there have been seven RCTs showing that hyperbaric oxygen chambers are beneficial for preventing amputation and promoting complete healing in patients with Wagner grade 3 or greater DFU[84,85]. In patients with Wagner grade 2 or lower DFU, there is inadequate evidence to justify the use of hyperbaric oxygen therapy as an adjuvant treatment. The most common adverse events associated with hyperbaric oxygen therapy are barotraumatic otitis, the inability to equalize middle ear pressure, and worsening of cataracts.

Topical growth factors

Epidermal growth factor (EGF), ascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) are polypeptide growth factors that have significant effects on tissue repair processes. These growth factors are released by platelets and activated macrophages that are required for normal wound repair[10,86]. Growth factors which are used topically were reported to reduce the incidence of lower limb amputation. Using of growth factors is essential to promote angiogenesis, enzyme production, and cell migration and proliferation.

PDGF is a crucial factor in wound healing and serves as a chemo-attractant for the migration of fibroblasts and neutrophils to the site of injury. It is the first recombinant growth factor approved for topical application of wound healing. Fewer RCTs evaluated the effectiveness of PDGF and all studies applied the topical gel with different concentrations[87,88]. The majority of them did not find significant healing improvements compared to the standard wound care.

FGF acts as a balance factor in the body and is important for tissue maintenance, repair, regeneration, and metabolism. FGF is a stronger angiogenesis factor than PDGF and VEGF. And FGF stimulates angiogenesis and proliferation of fibroblasts, forming granulation tissue. FGF has some limitations for the treatment of DFU wound healing, since it generally has a short half-life and require repeated administrations[89].

EGF is a wound modulator that is involved in cell migration and proliferation. Injecting EGF deep into the wound bottom and contours encourages a more effective pharmacodynamic response in terms of granulation tissue growth and wound closure[90,91]. EGF is perhaps the most widely used method in diabetic foot wound therapy but the results of studies are controversial or neutral. But our clinical experiences have shown that EGF is promising for healing foot ulcer (Figure 7).

Low level laser therapy

Low level laser therapy (LLLT) is a novel adjunctive therapy and is known to supply direct biostimulative light energy to body cells. This energy could stimulate molecules and atom of cells but does not cause a significant increase in tissue heat. Although different laser wavelengths have different depth of penetration of tissue, red laser has a deeper penetration than the others such as violet, blue, green, and yellow[92]. LLLT, which can be considered as a possible new treatment option for the diabetic foot, has a various effect on wound healing by cellular migration or penetration[93]. Otherwise clinical trials using human models do not provide sufficient evidence to establish the uselfulness and practical method in wound care regimes.

Although these newly adjunctive treatments have some benefits, they are costly and should be reserved for ulcers that fail to respond to standard treatments. Adjunctive treatment modalities should be considered as an addition to good wound care which must always include adequate off-loading and debridement therapy. Current evidence points towards VAC therapy and local stem cell application as an effective treatment than the other adjunctive modalities for diabetic foot healing. There is a need for well-designed blinded RCTs to determine the true efficacy of these interventions and to develop evidence-based practice guidelines.

PREVENTION OF FOOT ULCERATION

DFU are a devastating complication of diabetes mellitus. The mainstay of diabetic foot intervention is prevention. Preventative strategies in the form of education and regular foot assessments for peripheral vascular diseases and neuropathy along with risk stratification form the basis of the management of diabetic foot disease. Recently published guidelines highlight risk stratification for the assessment of risk for diabetic foot ulcer or risk of future amputation[55,94]: Very low risk: No loss of protective sensation and no peripheral arterial disease; Low risk: Loss of protective sensation or peripheral arterial disease or presence of callus formation alone; Moderate risk: Loss of protective sensation and peripheral arterial disease or presence of foot deformity; High risk: History of previous ulceration or previous amputation or renal replacement therapy or neuropathy and non-critical ischemic neuropathy with callus.

According to IWGDF prevention guidelines, it is recommended to examine a person who has a low risk of foot ulceration annually for signs or symptoms of loss of protective sensation and peripheral arterial disease. Patients with moderate or high risk should be screened every 3-6 mo. A person's risk status may change over time, thus requiring continual monitoring. Patients who have a risk of foot ulceration should be informed about controlling the whole surface of boot feet and the inside of shoes daily. Patients with moderate or high risk should be warned about wearing proper footwear to reduce plantar pressure. If there is a pre-ulcerative sign such as callus, appropriate treatment should be performed. Achilles tendon lengthening, joint arthroplasty, and single or pan metatarsal head resection may be considered for patients who cannot heal with non-surgical therapy.

If the preventative treatment modalities are carried out for patients with diabetes, the global patient and economic burden of diabetic foot disease can be considerably reduced. Decreasing the risk of ulceration also reduces the risk of infection, hospitalization, and lower extremity amputation in these patients.

CONCLUSION

DFU constitute a substantial burden for all over the world. Optimized therapy requires a collective

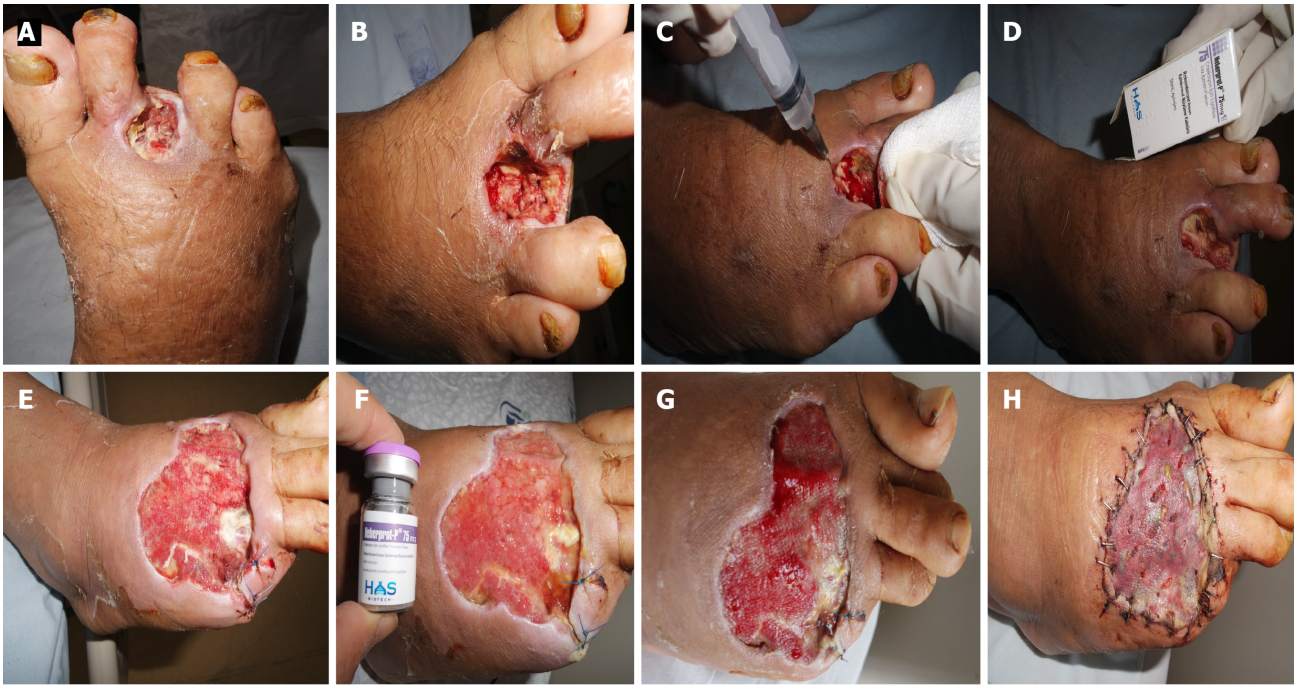

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Figure 7 Epidermal growth factor is perhaps the most widely used method in diabetic foot. A-D: Intralesional epidermal growth factor therapy into the wound bottom and contours encourages granulation tissue growth and wound closure; E-H: Before and after intralesional epidermal growth factor therapy.

refocusing on prevention and reallocation of resources from simply healing active ulcers. Multidisciplinary expert team is necessary for management of complex DFU and therefore multidisciplinary approach to patient care reduces the risk of amputation in patient with DFU. A combination of care from vascular, cardiovascular, infectious disease, and endocrinology disciplines as well as podiatrists and wound care specialists provides a full range of care for patients with DFU. Conventional therapies including debridement, off-loading, vascular assessment, and control of infection are principal treatment modalities. Otherwise, better outcomes could be obtained when the conventional treatment is combined with additional treatment in suitable patients.

FOOTNOTES

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MINIREVIEWS

Hyperbaric oxygen therapy and chemokine administration - a combination with potential therapeutic value for treating diabetic wounds

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Abstract

Non-healing wounds impart serious medical problems to people with diabetes. Amongst 15% of diabetic patients, the incidence of foot ulcer is the most prevailing, which confers a significant risk of limb amputation, mainly due to hypoxia and impairment in cell signaling. Alteration in the expression of chemokines and the related factors in diabetic conditions delays the recruitment of different cell types, including fibroblasts, keratinocytes, and immune cells such as macrophages to the site of injury, further impairing neovasculogenesis, re-epithelialization, and extracellular matrix formation. Thus, proper activation of effector cells through an accurate signal pathway is necessary for better therapeutic application. Hyperbaric oxygen therapy (HBOT) is the current treatment prescribed by medical practitioners, shown to have increased the wound healing rate by reducing the need for significant amputation among the diabetic population. However, the risk of morbidity associated with HBOT needs complete attention through rigorous research to avoid adverse outcomes. Altering the level of pro-angiogenic chemokines may regulate the inflammatory response, further promote vascularization, and enhance the complete healing of wounds in diabetic patients. Thus, a combination of better therapeutic approaches could pave the way for developing a successful treatment for diabetic foot and wound healing.

Key Words: Diabetic foot; Wound healing; Hyperbaric oxygen therapy; Chemokines; Combinatorial therapy

Core Tip: Diabetes induces slow or non-healing of wounds, increasing the risk of developing infection and other complications. Proper management of blood sugar levels is essential to improve the overall health. Hyperbaric oxygen therapy (HBOT) enhances the efficacy of wound healing rate in chronic diabetic foot ulcer patients. However, the systemic and meta-analysis data contradicts in cases associated with ischemic wounds. Also, the uncordial functioning of effector cells due to the interrupted signaling pathway involving chemokines and related growth factors worsens the condition of wound healing to a greater extent. Thus, a combinatorial approach of HBOT and chemokine administration could have potential therapeutic value for treating diabetic wounds with the existing clinical protocol.

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INTRODUCTION

Diabetic patients often encounter non-healing or improper healing of wounds, which is a serious medical problem. Amongst many complications imparted by diabetes, the incidence of diabetic foot ulcer (DFU) is the most prevailing, significantly increasing the risk of limb amputation in 25%-90% of diabetic patients if the proper medication is either not provided or followed[1]. It has been estimated that the current incidence of DFU will affect 15% of all patients related to diabetes[2]. DFU is an open sore or wound that generally begins from minor trauma, pressure, or irritation at the bottom of the foot. The morbidity of DFU leads to chronic pain, suffering, and poor quality of life for diabetic patients. The changes in the biomechanics of bones and architecture of soft tissues increase the risk of atherosclerotic arterial diseases and peripheral neuropathy, which could lead to nonenzymatic glycation predisposes causing ligament stiffness and decreased nerve sensation. Due to this, the patient would be unaware of pain on the foot or lower limb[3]. Also, prolonged hyperglycaemia impairs the function of immune cells, making the wound prone to infections. Thus, the overall physiological impairment associated with DFU complicates wound healing and detains precise treatment due to the lack of evidence-based protocol[4]. Although trauma and neuropathy are the critical triads for developing DFU, studies have shown that an intricate signaling mechanism is involved at the molecular level[5].

Wound healing is a cellular response which involves numerous processes such as hemostasis, inflammation, keratinocyte proliferation, angiogenesis, vascular epithelialization, fibroblast differentiation, collagen production, and tissue remodeling. However, oxygen perfusion to the site of injury is crucial for an effective outcome. Hypoxia, a state of low oxygen tension, induces cellular stress through a complex cascade by delaying the recruitment of pro-inflammatory cells, impairing growth factor expression, and resulting in defects in angiogenesis and extracellular matrix (ECM) formation[6]. Evidence suggests that diabetes induces hypoxia in the tissues of the kidneys, retina, adipose, and skin-related wounds[7]. Hypoxia-inducible factor (HIF) is the key transcriptional regulator that plays a prime role in the adaptive response to oxygen homeostasis. In the presence of oxygen (optimal concentration), HIF undergoes hydroxylation and subsequent ubiquitination to degrade in a shorter time. However, under hypoxic conditions, HIF undergoes stabilization and translocates to the nucleus to regulate the activation of genes associated with glucose metabolism and angiogenesis[8]. Studies showed that hyperglycemia destabilizes HIF and dysregulates downstream transcriptional activation, resulting in disease progression. However, the exact mechanism is still unknown[7].

In hyperbaric oxygen therapy (HBOT), a patient is treated by delivering 100% oxygen under a supra-atmospheric pressure. Evidence suggests that providing HBOT to patients suffering from Wagner grade 3 wound or higher DFUs during the postoperative period has greatly reduced the risk of limb amputation and incomplete re-epithelialization[9]. However, the clinical practice guidelines recommend against using HBOT for patients with Wagner grade 2 or lower DFU as the chance of oxygen toxicity is higher[10]. Thus, more research-based evidence is needed for effective treatment to prevent morbidity and mortality. Although HBOT is currently approved and recommended by the Centre for Medicare Studies for treating DFUs, the management remains complex[9].

Chemokines are signal molecules that play a crucial role in coordinating the activation and migration of immune cells to the site of injury[6]. The cytokines and growth factors produced by the immune cells promote wound healing during the inflammation and proliferation stage. Thus, an imbalance in the micro-environment will alter the network of their functionality, which could lead to prolonged healing or excess scar formation[11]. The expression profile shows that monocyte chemoattractant protein-1 (MCP-1) production by keratinocytes is significantly high in normal wound healing[12]. However, an *in vivo* study revealed that the reepithelization is delayed in MCP-1 deficient mice, indicating that the

dysfunction of the chemokine-dependent pathway could impair tissue remodeling[13]. Since chemokines are small proteins that do not have major modification regions other than two disulphide bonds, they are highly stable and can be used as adjuvants corresponding to their functional groups for wound therapy. Also, the ability of chemokines to bind G-protein-coupled receptors increases their likelihood as therapeutic targets for regulating biological activity, thereby mitigating disease progression[14]. This review examines chemokines/their specific receptors as potential targets for treating DFU and emphasizes the possible regulation to attain with HBOT for a combinatorial therapeutic approach to hasten the healing process.

Oxygen in wound healing

Oxygen is essential for maintaining basic cellular functions such as ATP production, protein synthesis, and reactive oxygen species (ROS) formation. ROS are oxygen molecules in a reduced format that are highly reactive. These radical derivatives are not only involved in the oxidative killing of bacteria but also act as secondary signal molecules[15]. Most well-known ROS molecules such as $\cdot O_2^-$, $\cdot O_2^{-2}$, H_2O_2, $\cdot OH$, and $\cdot OH^-$ are produced during oxidative phosphorylation. Like ROS, the reactive nitrogen species (RNS) are normal physiological by-products based on nitrogen oxidation that react mainly with thiols and transition metals to form nitrosyl-metal complexes. Cells such as macrophages, platelets, keratinocytes, and macrophages utilize these radical complexes as a signal response during wound healing[16]. However, their respective role in the cell cycle, homeostasis, cell-mediated defense, and activation of pro-apoptotic proteins for cell death significantly differs based on low, basal, high, and excessive concentrations. *In vivo* studies have shown that an optimal and sustained level of ROS mediates the secretion of pro-inflammatory cytokines and induces the matrix of metalloproteases. On the contrary, the addition of excessive reactive species (either ROS or RNS) was found to damage the ECM and diminish the function of dermal fibroblasts and keratinocytes. This shows that the lower and higher level of radical species has respective accelerating and decelerating effects on wound repair[17]. Thus, maintaining a balance in the level of oxidative species is critical for bringing effective outcomes.

Hyperglycaemia and oxidative stress

Diabetes is known to be accompanied by increased cellular oxidative stress. However, recent studies have only shown that hyperglycaemia resulting from unregulated blood glucose levels causes dysfunction in the antioxidant defense system by triggering the overproduction of ROS[18]. The hyperglycaemia-induced cellular damage is mainly associated with: (1) Excessive formation of advanced glycation end products; (2) Protein kinase C activation; and (3) Increased polyol and hexosamine pathway flux, all of which could enhance oxidative stress[19]. The exact mechanism is ambiguous as one influences the other. Still, the prevailing evidence suggests that hyperglycaemia increases the availability of electron transfer donors such as $FADH_2$ and NADH, which increases the electron flux, further creating hyperpolarization of mitochondrial membrane potential due to a change in ATP/ADP ratio. The high electrochemical potential difference leads to the partial inhibition of electron transport between coenzyme Q and complex III, resulting in electron accumulation, further driving the partially reduced O_2 to generate free radical anion superoxide, and thus impairing the cell function[20].

Circulatory endothelial progenitor cells (EPCs) produced in the bone marrow play a significant role in the regeneration of the endothelial lining of blood vessels in response to ischemic conditions[21]. The antioxidant enzyme level of EPCs was found to be enhanced in a low oxygen environment to engraft vasculogenesis. However, their activation and proliferation are significantly impaired in an oxidative stress environment and the baseline pattern is similar to that of diabetic conditions[22]. Other than activation and proliferation, the migration of EPCs to the injured sites followed by tissue homing based on chemokine signal is important for wound repair. Nevertheless, the process is diminished in diabetic condition due to signal deficit, further impairing EPC function[23]. Thus, the elevated level of ROS production is believed to be the prime factor by which hyperglycaemia-mediated diabetes affects the normal wound healing process.

HBOT and oxidative response in diabetic wound healing

The pathological state of delayed wound healing is associated with prolonged oxygen deficit. The increase in the amount of oxygen would generate a favorable gradient for its diffusion into the affected tissues[3]. The management of chronic non-healing wounds by HBOT increases the rate of oxygen perfusion 10-50 folds and shows a correlation by modulating the inflammatory response with an increase in ROS production[24]. Vascular endothelial growth factor (VEGF), a key angiogenic factor responsible for maintaining blood vessel integrity, is stable under hyperoxia and hypoxia conditions. The function of endothelial-1, an endogenous vasoconstrictor responsible for the maintenance of blood pressure and basal vascular tone, seems to have significantly decreased under hyperoxic conditions[25]. Thus, it is paradoxical to perceive that the increase and decrease in oxygen concentration have alternative effects on blood vessels together with varying levels of ROS production, thus imparting a setback on HBOT.

A systematic study based on 9000 previous records on the effect of hyperoxygenation shows that HBOT increases the level of oxygen radicals and increases the chance of inducing oxidative stress. On the contrary, the meta-analysis data reveals that HBOT stimulates the release of angiogenesis-promoting cytokines and growth factors, whose function is impaired when oxidative stress is high, as in the case of diabetes[26]. The most remarkable understanding is obtained from the thermal imaging data of an HBOT-treated wound with decreased temperature, indicating a decline in inflammation[27]. Since no significant difference in the profile of anti-inflammatory markers was observed in HBOT, its direct role in anti-inflammation seems less probable. Thus, promoting a wound to an anti-inflammatory state from a prolonged inflammatory condition (where ROS level is high) could be possible by regulating a nuclear factor that suppresses the pro-inflammatory genes. Nuclear factor kappa B (NF-κB) is a critical transcriptional factor in inflammation that activates several pro-angiogenic genes together with HIF-1α under hypoxia conditions. However, inhibitor of kappa B alpha (IκBα), another nuclear factor that is stimulated under hyperoxic conditions, inhibits NF-κB, resulting in the downregulation of pro-inflammatory transcription factors and pushing the cellular environment towards an anti-inflammatory state [28]. HBOT could establish the same condition in the wound micro-environment despite oxidative stress and aids healing. An *in vivo* study validates that hyperoxia induced during HBOT session is associated with decreased NF-κB expression and stimulated the activation of IκBα, which is generally degraded under hypoxia[29]. Although it seems promising, no significant evidence is available about the cellular damage induced by the preformed oxidative species or its reversal effect by HBOT before the establishment of anti-inflammatory phase, which needs to be addressed through research for regulating the interventional procedure.

Macrophage polarization in normal vs diabetic wound

Macrophage infiltration on the wound site is mainly derived from the monocyte precursors in response to pathogen-associated modifying proteins or damage-associated modifying proteins. Besides the scavenging activity, macrophages play other roles in tissue regeneration and wound repair[30]. However, depending on the phenotype, their functionality is assigned in the tissue micro-environment.

The macrophages are divided into three subgroups based on the markers that they express on the surface: Classical macrophages ($CD14^{++}CD16^{-}$), intermediate macrophages ($CD14^{++}CD16^{+}$), and non-classical macrophages ($CD14^{++}CD16^{++}$)[31]. Classical macrophages are known as M1 or pro-inflammatory macrophages that are triggered by tumor necrosis factor (TNF) and lipopolysaccharides and produce pro-inflammatory cytokines such as interleukin (IL)-12 and IL-23, together with ROS. The non-classical macrophages are known as M2 or resolving macrophages that are stimulated by the anti-inflammatory cytokines such as IL-4 and IL-10 to activate the release of growth factors such as transforming growth factors and insulin-like growth factors[32]. In normal wound healing, M1 predominates up to 3 d in clearing up the pathogens and dead/dying cells from the wound site and causes inflammation. The transition to M2 occurs thereafter with a peak in activity on day 7, promoting wound healing. Studies have shown that impairment in the transition to M2 and persistent polarization of M1 macrophages are responsible for prolonged wound healing in chronic disease conditions[31]. An *in vivo* study showed that the ratio of CCR7-CD48, a respective chemokine receptor and marker found on M1 and M2 macrophages, is higher in diabetic mice with impaired wound healing than in controls, indicating the dysfunctionality of macrophage switching/transition and its importance in wound repair. Also, it has been shown that the prolonged M1 polarization reduces the expression of matrix metalloproteinases together with increased secretion of pro-inflammatory cytokines such as TNF-α, affecting the keratinocyte migration and leading to the concussion of normal wound healing process[33]. Several factors were found to contribute to the persistent polarization of M1 macrophages, which is why the therapeutic development could be focused on either blocking the inflammatory cascade activating the M1 phenotype or promoting M2 transition to resolve the debilitating diabetic chronic wounds.

Chemokines - a potential regulator of macrophage polarization and wound differentiation

Chemokines are a family of secretory proteins with low molecular weight (8-12 kDa) that have a prominent role in chemotaxis and activation of immune cells. The four subfamilies of chemokines C, CC, CXC, and CX3C are classified based on the two conserved cysteine residues present at the N-terminal motif[34]. Chemokines are important in regulating angiogenesis during hemostasis and the inflammatory phase of wound healing for clot formation and the influx and efflux of migratory cells. Also, they control the formation and regression of neovessels during the proliferation and remodeling phase to assist the healing wound in meeting the metabolic need and scar formation. Thus, playing a pivotal role in orchestrating the precise sequence of events, chemokines are crucial in all stages of wound healing (Table 1)[35]. As discussed earlier, for the establishment of the proliferation phase, the pre-formed inflammation in the tissue microenvironment should get declined by the anti-inflammatory signal cascade to establish the transition of the M1 to M2 phenotype to aid tissue repair.

Adipose tissue macrophages constitute 10%-15% of the total cell population in healthy individuals, and they predominantly show M2 phenotype with high insulin sensitivity. However, in obesity, the adipocytes secrete pro-inflammatory markers that trigger the recruitment of monocytes *via* the CCL5-CCR5 pathway. The macrophages derived from those monocyte precursors acquire the M1 phenotype and contribute to prolonged inflammatory environments[36]. On the contrary, the intrahepatic

Table 1 Notable chemokines and their function at different stages of wound healing with their relative expression in normal *vs* diabetic condition

Chemokine	Stage	Function	Role	Relative expression in normal wound	Relative expression in diabetic wound
CXCL4/platelet factor 4	Hemostasis	Angiostatic	Inhibition of VEGF-induced VEC proliferation	+++	+
CCL2	Inflammation	Macrophage recruitment	Activation of P38MAPK pathway	+++	++++
CCL5	Inflammation	Eosinophil recruitment	PKB phosphorylation to induce apoptosis	+++	++
CXCL8	Hemostasis & inflammation	Neutrophil recruitment	Phagocytosis	+++	+
CCL3	Proliferation	Macrophage polarization	ECM formation	+++	Unknown
CXCL11	Proliferation	Angiostatic	Basement membrane regeneration	+	Unknown
CXCL12	Proliferation	Granulation tissue formation	Unknown	++	NA
CCL2	Proliferation	Type-I collagen deposition	Upregulation of MMP-1	+++	NA

+: Denotes minor increase in expression; ++: Denotes moderate increase in expression; +++: Denotes large increase in expression; NA: Not available; CXCL: chemokine (C-X-C motif) ligand; CCL: Chemokine ligands; VEGF: Vascular endothelial growth factor; VEC: Vascular endothelial cells; MAPK: Mitogen activated protein kinase; PKB: Protein kinase B; ECM: Extra cellular matrix; MMP: Matrix metallopeptidase.

monocytes in the presence of the anti-apoptotic protein BCL2 are mediated by the CX3CL1-CX3CR1 pathway and show a less inflammatory phenotype characterized by decreased TNF-α and nitric oxide synthase production[37]. Thus, macrophage polarization in metabolic disorders could be modulated by regulating the chemokines and their specific receptors.

Recent advancement in stem cell-based approaches has garnered significant interest as they have potential therapeutic value. Studies have shown that exosomes derived from mesenchymal stem cells (MSCs) possess immunomodulatory effects that can induce the transition of pro-inflammatory macrophages to anti-inflammatory phenotype in various inflammatory-associated disease conditions [38]. These MSC-derived exosomes exhibit high expression of angiogenic and tissue repair factors such as VEGF, extracellular matrix metalloproteinases, and matrix metallopeptidase 9[39]. The adipocyte-derived MSCs that express Arg-1 and IL-10 based on the activation of STAT3 transferred by the exosomes are shown to alleviate inflammation through M2 polarization[40]. The genes present downstream of macrophage transcriptional factors, such as interferon regulatory factor/STAT, which arranges the polarization. Bruton's tyrosine kinase with STAT1/STAT5 and Kruppel-like factor 4 with STAT3/STAT6 induce the polarization of the M1 and M2 phenotype, respectively[41,42]. However, for the complete induction of the anti-inflammatory phenotype, the peroxisome proliferator-activated receptor gamma is essential, and its absence diminishes insulin sensitivity, further resulting in hyperglycaemia that impairs cellular function[43].

Other than MSCs, epidermal stem cells aid in tissue repair by modulating the migration and proliferation of EPCs to the injury site. Abnormal EPC migration and a lack of tubularization cause impaired angiogenesis in diabetic patients[21]. Stromal cell-derived factor 1 (SDF-1), a chemokine belonging to the CXC family, recruits EPC to the wound site by interacting with CXCR receptors 4 and 7. A study has shown that the expression of SDF-1 between acute and chronic wounds differs significantly. In the case of chronic wounds, no influence was observed with EPC migration, thus, an exogenous administration of SDF-1 is inevitable to accelerate the wound healing rate[44]. However, the duration of the chemokine gradient and its bioavailability are essential factors to consider in the effectiveness of the wound-healing rate. Instead of a single dose administration, a formulation that enhances a slow release of the element might have a significant positive effect on tissue regeneration. To achieve this, a biomaterial scaffold that retains the bioactivity of chemokine could be developed for tissue engineering purposes. SDF-1 encapsulated in poly(ethylene glycol citrate-*co*-N-isopropyl acrylamide) has improved the tissue healing rate in diabetic mice with sustained release of the factor for up to 3 wk without any burst[45]. Modifying the hydrogel systems, such as integrating anti-oxidant properties, could render more advantages for rapid healing, and developing such state-of-the-art techniques could revolutionize the therapeutic aspects of treating chronic diabetic wounds.

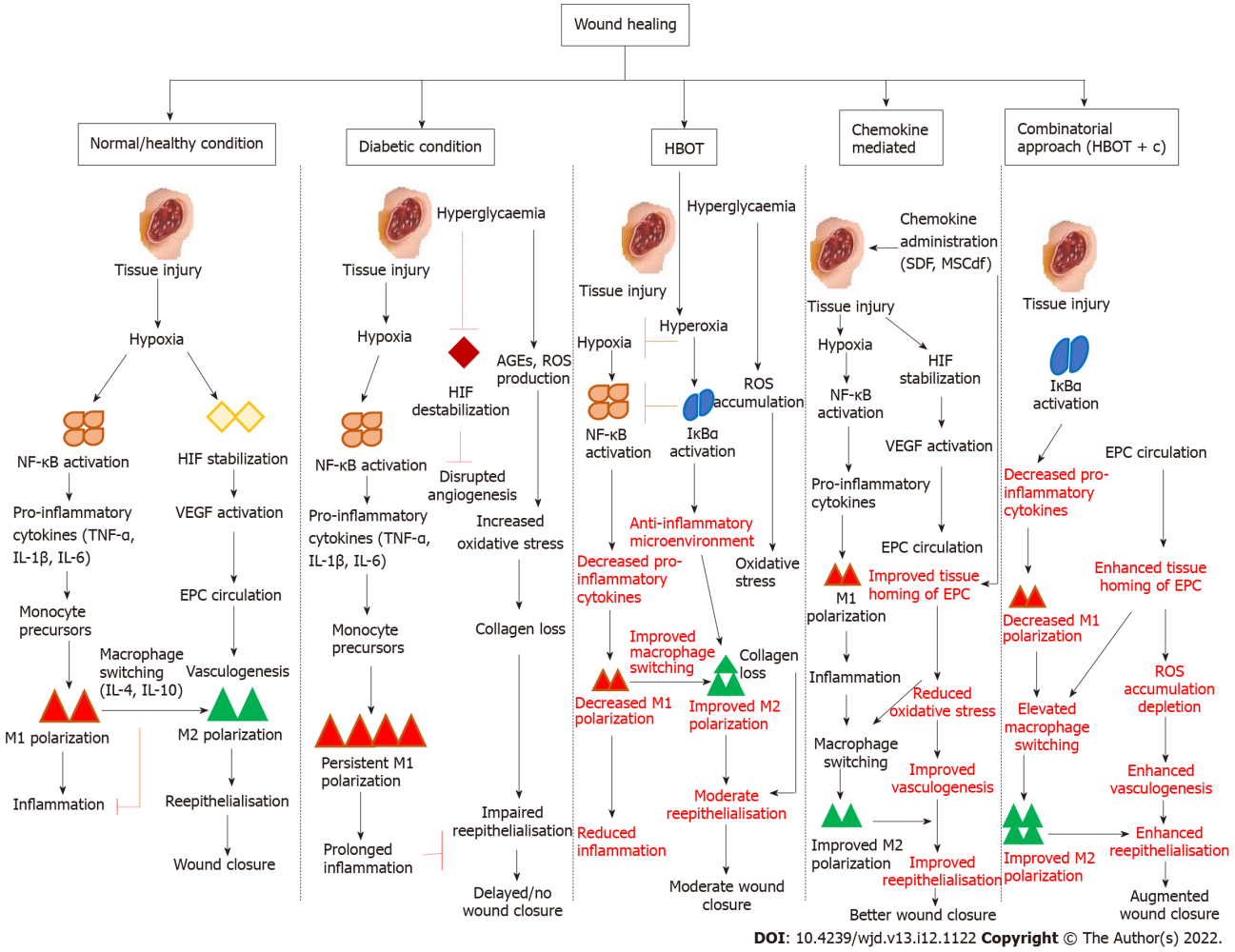

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Figure 1 Mechanism of wound healing in normal *vs* diabetic conditions and plausible molecular regulations achieved by hyperbaric oxygen therapy, chemokine administration, and combinatorial approach (hyperbaric oxygen therapy + chemokine administration) for augmented therapy. HBOT: Hyperbaric oxygen therapy; SDF: Stromal cell-derived factor; MSC: Mesenchymal stem cell; AGEs: Advanced glycation end products; ROS: Reactive oxygen species; HIF: Hypoxia-inducible factor; VEGF: Vascular endothelial growth factor; EPC: Endothelial progenitor cell; NF-κB: Nuclear factor kappa B; TNF: Tumor-necrosis factor; IL: Interleukin; IκBα: Inhibitor of kappa B alpha.

CONCLUSION

Diabetes is a chronic disease that brings delirious effects through prolonged inflammation that could lead to other metabolic disorders such as cardiovascular diseases, hypertension, and renal diseases. Several interventions have been suggested, including a healthy diet, exercise, and proper medication to lessen the adverse outcomes. However, a better therapeutic approach is needed for an effective outcome despite the standard procedures. The problem with delayed wound healing and persistent infection in diabetic patients is attributed to the deficiency of oxygen perfusion to the injured site. The resulting hypoxic environment alters the sequence of cellular events from the normal wound healing and complicates the process further. HBOT is found to fasten the wound healing rate in DFU cases by inducing angiogenic factors and other critical components of the cellular cascade. Although HBOT is found to be efficient in reverting the ischemic condition of the wound, complete reliance on the interventional procedure is not enough, as wound healing is a multifactorial process. Thus, the efficiency of chemokine-mediated response is essential for activating the effector cells that participate in wound healing.

The combinatorial therapeutic approach could be of interest as it will likely lead to a better outcome. HBOT and simultaneous administration of tissue-specific chemokine/receptor modulating factors could overcome multiple wound healing deficits observed in diabetic conditions (Figure 1). Since not much research was carried out earlier with the proposed combination, this review emphasizes the researchers to conduct various controlled trial studies with Food and Drug Administration-approved biologics to explore the potential and developing novel strategies and better clinical practices for treating diabetic wounds.

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MINIREVIEWS

The role of artificial intelligence technology in the care of diabetic foot ulcers: the past, the present, and the future

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Abstract

Foot ulcers are common complications of diabetes mellitus and substantially increase the morbidity and mortality due to this disease. Wound care by regular monitoring of the progress of healing with clinical review of the ulcers, dressing changes, appropriate antibiotic therapy for infection and proper offloading of the ulcer are the cornerstones of the management of foot ulcers. Assessing the progress of foot ulcers can be a challenge for the clinician and patient due to logistic issues such as regular attendance in the clinic. Foot clinics are often busy and because of manpower issues, ulcer reviews can be delayed with detrimental effects on the healing as a result of a lack of appropriate and timely changes in management. Wound photographs have been historically useful to assess the progress of diabetic foot ulcers over the past few decades. Mobile phones with digital cameras have recently revolutionized the capture of foot ulcer images. Patients can send ulcer photographs to diabetes care professionals electronically for remote monitoring, largely avoiding the logistics of patient transport to clinics with a reduction on clinic pressures. Artificial intelligence-based technologies have been developed in recent years to improve this remote monitoring of diabetic foot ulcers with the use of mobile apps. This is expected to make a huge impact on diabetic foot ulcer care with further research and development of more accurate and scientific technologies in future. This clinical update review aims to compile evidence on this hot topic to empower clinicians with the latest developments in the field.

Key Words: Diabetic foot ulcers; Photographic monitoring; Artificial intelligence technology; Digital photography; Mobile app; COVID-19 pandemic

Core Tip: Diabetic foot clinics faced major challenges during the COVID-19 pandemic due to lockdowns and social distancing measures as a significant proportion of patients were unable to physically attend the clinics. This situation boosted the attempts for transition of face-to-face foot clinics to virtual clinics as in many other types of medical care during the pandemic. Monitoring of diabetic foot ulcers (DFUs) by digital photographic technology and mobile phone-based photography have revolutionized this area of clinical care in recent years and mobile apps are expected to accelerate this progress. This article reviews the past, the present and the future of artificial intelligence technology in the care of DFUs.

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INTRODUCTION

Foot ulceration caused by micro- and/or macrovascular disease is a common complication of diabetes mellitus (DM). The global prevalence of diabetic foot ulcers (DFUs) at any point in time is estimated to be 6.3%[1], with 25% of DM patients developing a DFU in their lifetime[2]. DFUs significantly increase morbidity [including lower extremity amputations (LEA)] and mortality among sufferers, and the care of DFUs poses very high healthcare expenditure worldwide. Approximately 50% of DFUs are associated with infections[3], and approximately 20% of moderate to severe DFU infections may lead to minor or major amputations[4]. DFU-related LEA is associated with a 10-year survival rate as low as 24% which is lower than that of several forms of cancers[5]. These alarming figures give us a broad outline of the health-related risks posed by DFUs.

Appropriate care of DFUs to ensure rapid cure and avoid complications involves regular monitoring of the progress of healing with periodic review of the ulcers, frequent dressing changes, antibiotic therapy for infection control, optimal control of DM, and adequate offloading of the ulcers to avoid ongoing damage due to pressure on the wound area. Diabetic foot clinics usually provide comprehensive DFU care by multidisciplinary specialist teams involving podiatrists, orthotists (who make appropriate footwear/devices for offloading the DFU), diabetes specialist nurses, and diabetologists. However, insufficient manpower, long clinic waiting lists, and logistic issues with patient transport to the clinics can all pose problems for timely review of patients with DFUs in the foot clinics. Manpower shortage resulting in longer waiting periods prior to review by the footcare team has been a major issue in ulcer care in diabetes foot services across the world in recent years[6,7]. Delays in presentation for foot ulcer review have been identified as an important reason for wound non-healing[8]. Complications of foot ulcers including amputations can be the devastating sequel of such delays in review by the footcare team.

The COVID-19 pandemic posed major challenges in DFU care across the globe due the above reasons [9-11]. Delays in ulcer reviews may be associated with detrimental effects on DFU healing that may even lead to amputations[10,11]. Telephone and video clinics during the pandemic period helped clinicians to address some of the issues related to the inability of patients to attend medical clinics during the COVID-19 lock-down period. Attempts to develop artificial intelligence (AI) algorithms by the scientific fraternity to enhance patient care through virtual clinics attained greater momentum in relation to the pandemic[12,13].

Monitoring the progress of DFUs by comparing serial ulcer photographs during the follow-up in foot clinics has been practiced by many diabetologists over the past 2-3 decades. Refinement of the photographic methods by sophisticated digital technology and mobile phone cameras have revolutionized DFU care in recent years[14-16]. Some patients often use mobile phone cameras to capture their foot ulcer photographs for self-monitoring wound progress and to help clinicians to understand the previous status of their wound during clinic review.

However, much more multidisciplinary scientific research input and output are necessary in this area. Further refinement of this rapidly advancing digital technology for optimal use in day-to-day clinical practice could result in better care of patients with DFUs. This review attempts to gather up-to-

date evidence to summarise the past, present, and future dimensions of AI algorithms to empower clinicians across the globe to appropriately utilise digital technology for DFU care.

PHOTOGRAPHIC MONITORING OF DFU PROGRESS

Diabetes care professionals often see several cases of DFU in their day-to-day clinical practice and often forget the previous ulcer grade, character and even the site during subsequent visits, weeks later when followed up in the foot clinic. Review of the previous photographs during subsequent follow-up visits should provide clinicians with a good clinical assessment of the progress of the ulcer and help prognostication[17]. Photographic monitoring also enhances appropriate continuity of care by different clinicians running the diabetic foot services during their review of the same case.

Important issues which can arise while comparing the photographs are the differences in the distance from which photographs are captured (can be largely avoided by placing a measuring tape on the ulcer while photographed), differences in the illumination of pictures from brightness of background light when the photographs were taken, and the likelihood of low-quality images without adequate focusing of cameras by individuals taking the picture. Photographs are usually taken without flashlight to avoid undue illumination that can reduce the picture quality. Clinical evidence suggests that prediction of ulcer healing is possible by regular photographic monitoring[17]. Figure 1 shows the photographic monitoring of the progress of a DFU at various stages.

HISTORY OF DIGITAL ARCHIVING OF THE DFU PHOTOGRAPHS

Use of digital cameras for photography has become a common practice since the early 1980s after the invention of digital photographic technology in 1975. Digital archiving of foot ulcer photographs in computer databases was a great leap forward in the technology for monitoring DFUs. Print outs of foot ulcer photographs are still used in remote settings where internet and computers are not freely available in clinical practice. This is in fact more expensive (costs incurred with colour printing and use of good quality photographic paper) and cumbersome in the modern era.

Fading of the colour print outs over time makes the situation worse regarding DFU monitoring using this method. A review of the serial images in the computer database of digital photographs makes the work of diabetes foot care professionals much easier when this facility is available[18]. Lack of degradation of image quality over time as in printed photographs is another great advantage of digital archiving of DFU images.

Mobile phones became popular for telephone communications globally in the early 2000s and newer versions of these devices with cameras and video recording facilities came into the market a few years later. With rapid advancements in technology, smartphone devices using high resolution cameras are now widely available and have become an integral part of modern life in the 21st century. Diabetes care professionals soon started using mobile phone cameras and video facilities for the monitoring and management of DFUs[19-21]. Some patients themselves were using mobile phones to obtain images of their DFUs prior to their foot clinic visits so that they could show the status of their ulcers earlier. Although the initial studies on use of mobile phones for monitoring DFUs were not very promising[22], subsequent studies show encouraging results[23-25].

INTEGRATING DFU PHOTOGRAPHS WITH COMPUTER-BASED DIGITAL TECHNOLOGY FOR DIAGNOSTICS

AI and its applications have been utilised in various branches of modern science and technology including the medical field over the past few decades to improve physical and intellectual human work output. Such technologies can also be utilised to monitor the progress in DFU care. DFU datasets were utilised for training and testing the machine learning processes. The collection of such datasets supports the ongoing research for DFU academic AI challenges, such as those hosted by the Medical Image Computing and Computer Assisted Interventions (MICCAI) conferences[26,27]. These challenges are used to promote and advance ongoing research and to increase exposure within the associated academic fields.

Machine learning algorithms have been found to be very useful in detecting DFUs with high accuracy rates in previous studies[28-30]. These algorithms are developed by using large datasets of images captured from the foot clinics. The development of computer-aided DFU diagnostic algorithms involves multiple stages such as pre-processing, feature extraction, detection, classification, and segmentation of DFU wounds[30]. These tasks can be challenging in real-world settings due to the low-quality of images from inadequate focusing, motion artifacts, inadequate lighting, and backlight, deformities in the foot/toes, size and shape of the ulcers (very small, very large and curved ulcers), and newly formed or

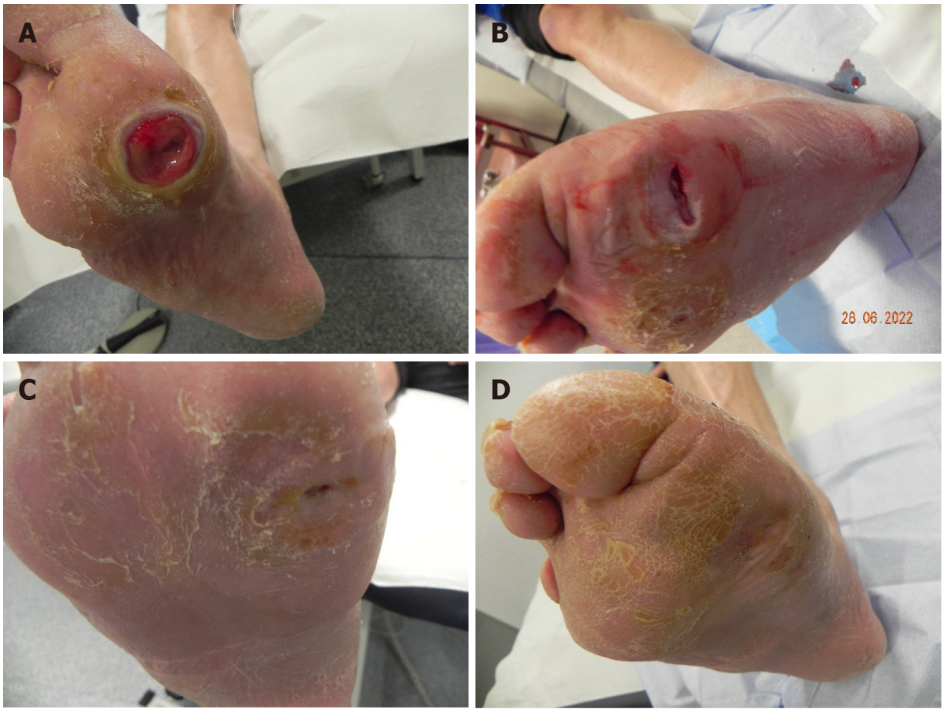

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Figure 1 Photographic monitoring of the progress of a DFU at various stages. A: Infected foot ulcer on April 25, 2022; B: After 2 mo of regular offloading and dressings (on June 28, 2022) with initial 3 wk of antibiotic therapy; C: Further improvement of ulcer on July 23, 2022: D: Complete healing of ulcer on August 22, 2022.

early ulcers which are easily missed during the capture of photographs in foot clinics[16].

The first and foremost step in the development of machine learning algorithms is the detection of DFUs from foot photographs. This task has been successfully carried out by previous researchers as mentioned above[28-30]. Further refinement of machine learning is currently being undertaken by incorporating the classification systems for DFUs, as in the real-world settings, to promote AI-based diagnostics and prognostication. Several manual DFU classification systems are currently used by foot care professionals such as Wagner, University of Texas, and SINBAD (Site, Ischaemia, Neuropathy, Bacterial infection, Area, and Depth) for DFU monitoring and management[30]. These manual approaches may benefit from the automated processes afforded by AI.

Incorporation of such complex features in the AI-technology for day-to-day clinical practice to enhance diagnosis and prognostication can be challenging. These challenges include: (1) The significant time-burden involved in the DFU image data collection and appropriate labelling; (2) The inter- and intra-class variations depending on differences in the classification of DFUs; (3) Lack of standardization of DFU datasets (caused by camera distance from the foot, image orientation and lighting conditions); and (4) The differences in ethnicity, age, sex, and foot size of the patients[30]. Development of deep learning AI algorithms requires large-scale datasets for automated DFU analysis to reproduce comparable results to those by experts. Researchers currently working in isolation may not achieve reproducible research outputs. Large DFU datasets used for training and validation by multiple professionals from different institutions across the globe should help to refine these pitfalls in machine learning algorithms for DFU classification and diagnosis. To enable innovation from clinicians and researchers, Yap *et al*[26] proposed the diabetic foot ulcer challenges by providing the publicly available datasets, for comprehensive evaluation of object detection frameworks on DFU detection using convolutional neural networks trained on the DFUC2020 dataset[15]. Examples of manually delineated DFU photographs from the training set are shown in Figure 2. Morphological classification of DFUs into different types (such as infection, ischaemia, both, and none) is carried out in these datasets to enable machine learning.

SERIAL DEMARCATION OF ULCER PHOTOGRAPHS TO ASSESS PROGRESS OF DFU HEALING

At present, monitoring the healing process of DFUs is largely completed by regular patient follow-up visits in the multidisciplinary foot clinics[31-33]. Since 2020, the COVID-19 pandemic has made a huge impact on DFU care across the world due to the issues related to lockdowns and social distancing

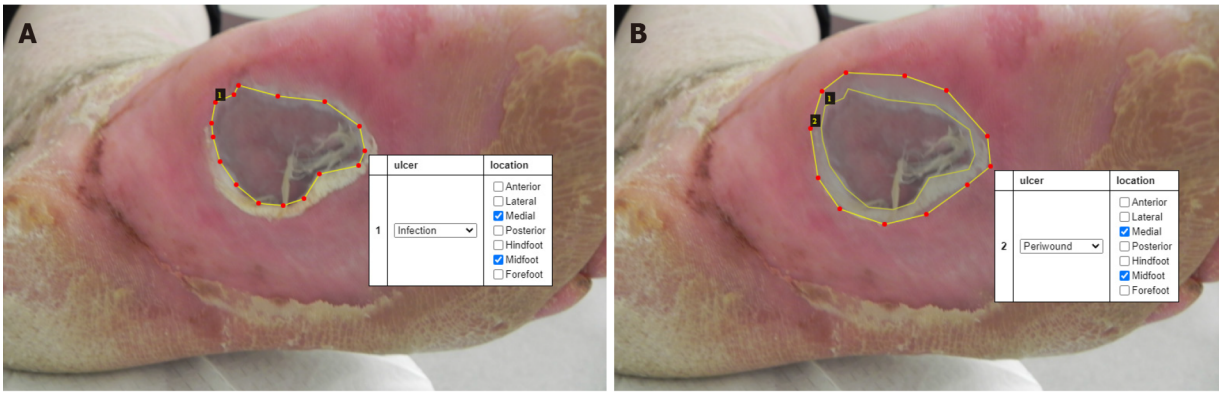

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Figure 2 An infected ulcer on the plantar aspect of the left foot. A: The ulcer is labelled (dotted line marking the boundaries) for the training set. The white box shows the site and type of ulcer. B: The peri-wound of the ulcer is labelled with a dotted line. The white box shows the site of the ulcer and the peri-wound.

measures enforced by governments to curtail human devastation as a result of the global health emergency. Foot clinic attendance[34] and hospitalisations for DFUs[35] were significantly reduced during the pandemic. Although worsening of DFUs was comparatively less owing to reduced outdoor human activity[36], many patients presented to clinicians late, increasing the risk of complications from DFUs[35,37]. The pandemic reinforced the urgent necessity to develop AI-based wound care algorithms for remote monitoring of DFUs.

To enable the development of machine learning algorithms, serial demarcation of DFUs is important to assess the progress of the healing process along with wound classification based on one of the above-mentioned standard systems. Therefore, characterisation of DFUs requires demarcation of wounds at various stages in the datasets by experts in the field after which the data can be used for training, validation, and testing. This task is highly labour-intensive and requires huge DFU training and validation datasets.

PHOTOGRAPHS ACQUIRED BY MOBILE PHONE CAMERAS FOR DFU MONITORING

New generation mobile phones can capture high resolution DFU photographs comparable to commercial digital cameras. Many patients already use mobile phone photography for self-monitoring their DFUs[14,22]. Some patients serially document their wound progress using these images and meticulously bring them to foot clinics during their follow-up visits for DFU care. Occasionally, patients send their photographs to footcare professionals electronically or as email attachments. Although safety issues related to secure transfer of such clinical information without breach of confidentiality can be challenging at present, wider use of mobile phone photography for self-monitoring of DFUs progress might revolutionise future foot care.

DEVELOPMENT OF MOBILE APPS FOR DFU MONITORING

Various mobile phone apps are currently used worldwide in many domains of daily activities to improve the quality of human life. Such mobile apps for DFU monitoring and care are under development. Cassidy *et al*[38] developed the first mobile app capable of accurate DFU detection using AI and cloud-based technologies. This system was tested in a 6-mo clinical evaluation at two UK National Health Service hospital sites (Lancashire Teaching Hospitals and Salford Royal Hospital) and is currently being further developed to improve functionality and accuracy. Additional app features, such as automated classification of DFU wound pathology[39] and automated delineation of wound/peri-wound regions are also being investigated to provide a more clinically relevant system.

These new technological advancements are expected to revolutionise remote wound care by enabling patients to self-monitor their DFUs and contact clinicians when they find any concern or deterioration of their disease. This would also enhance flexibility in the functioning of foot clinics by reviewing and triaging the most appropriate DFUs to be seen in the clinics by remote monitoring of patients.

PREDICTION OF DFU PROGRESS BY INTEGRATION OF CLINICAL AND BIOCHEMICAL PARAMETERS AND ULCER PHOTOGRAPHS

Although regular wound care with dressings, appropriate antibiotic therapy for infections, offloading the ulcers to relieve pressure-related delay in healing and revascularisation of the ischaemic foot are the cornerstones in the management of DFUs, several other clinical (comorbid conditions such as renal disease, heart failure, and immunosuppressed states) and biochemical parameters (such as hyperglycaemia, anaemia, and high haemoglobin A1c) may impact the DFU healing process[40-44]. Integration of these clinical and biochemical parameters into machine learning algorithms should help us to develop prediction models using AI technology.

We note however that the development of such algorithms is much more labour-intensive as demarcation exercises to develop deep learning models require larger datasets with clinical and biochemical parameters of individual patients incorporated within the neural network. However, appropriate use of computer technology integrated with digital applications can help to reduce the physical burden on researchers in developing such models.

FUTURE PERSPECTIVES

Recent research has investigated the utilisation of patient data in the training of deep neural networks in various medical imaging domains[45]. These studies indicate that machine learning models trained on patient data can be used to boost the performance of convolutional neural networks trained on wound/lesion images. Research of this nature is ongoing and represents a way to incorporate a more integrated method of wound analysis that considers multiple data points. The development of mobile apps integrating such new advancements in technology is expected to revolutionise the global scenario of DFU care in the near future.

CONCLUSION

Digital applications in the daily management of DFUs have evolved rapidly in recent years to a level of remote diagnosis and monitoring of wounds in community settings. The COVID-19 pandemic has accelerated research and development of such innovative technological applications in the past two years. Photographic monitoring of foot ulcers has been practiced in many centres across the world in the past few decades providing DFU care. The invention of digital photographic technology in 1975 further boosted DFU care because of the ease of electronic archiving of ulcer images during clinical follow-up. Photography using mobile phone cameras has become a huge leap forward in this direction in recent years empowering patients and clinicians to further improve DFU care.

AI-based digital algorithms are currently being developed rapidly through collaborative global effort between AI experts and clinical teams. Mobile camera-based digital technologic applications are under development to enhance remote diagnosis, monitoring, and follow-up care of DFUs. Prediction models of wound healing are also under development now making use of linking the ulcer characteristics of DFU images to the clinical and laboratory parameters of diabetic patients. These collaborative efforts between clinicians and computer scientists across the world should revolutionise such discoveries to empower diabetic foot patients to self-monitor and manage their DFUs to a greater extent.

FOOTNOTES

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MINIREVIEWS

Single nucleotide variations in the development of diabetic foot ulcer: A narrative review

Yan-Jun Hu, Chen-Sheng Song, Nan Jiang

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Abstract

Diabetes mellitus has become a global health problem, and the number of patients with diabetic foot ulcers (DFU) is rapidly increasing. Currently, DFU still poses great challenges to physicians, as the treatment is complex, with high risks of infection, recurrence, limb amputation, and even death. Therefore, a comprehensive understanding of DFU pathogenesis is of great importance. In this review, we summarized recent findings regarding the DFU development from the perspective of single-nucleotide variations (SNVs). Studies have shown that SNVs located in the genes encoding C-reactive protein, interleukin-6, tumor necrosis factor-alpha, stromal cell-derived factor-1, vascular endothelial growth factor, nuclear factor erythroid-2-related factor 2, sirtuin 1, intercellular adhesion molecule 1, monocyte chemoattractant protein-1, endothelial nitric oxide synthase, heat shock protein 70, hypoxia inducible factor 1 alpha, lysyl oxidase, intelectin 1, mitogen-activated protein kinase 14, toll-like receptors, osteoprotegerin, vitamin D receptor, and fibrinogen may be associated with the development of DFU. However, considering the limitations of the present investigations, future multi-center studies with larger sample sizes, as well as in-depth mechanistic research are warranted.

Key Words: Diabetic foot; Diabetic foot ulcer; Diabetic foot osteomyelitis; Single nucleotide variations; Narrative review

Core Tip: The pathogenesis of diabetic foot ulcer (DFU) is complex and is associated with both extrinsic and intrinsic factors. Most previous studies have reported the roles of external factors in DFU development and have neglected internal factors. In this narrative review, we focused on single-nucleotide variations (SNVs), as a representative of host factors. We summarized recent findings regarding the relationships between genetic SNVs and susceptibility of different populations to DFU. Future multicenter investigations with larger sample sizes, as well as in-depth mechanistic research, are necessary to better recognize and understand the roles of SNVs in DFU pathogenesis.

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INTRODUCTION

Diabetes mellitus (DM), one of the most frequently encountered metabolic disorders, has become a global health problem and is considered a public health emergency[1]. The severity of DM is not only attributed to the disorder itself but also to its associated complications, influencing both life expectancy and quality of life[2]. DM-related complications affecting the lower extremities are common, complex, and costly ("3Cs"), with diabetic foot ulcer (DFU) being the most frequently recognized type[3]. It is estimated that the lifetime incidence of DF or DFU is approximately 15%–25% among patients with DM [4,5]. DFU remains one of the most challenging disorders for physicians to treat, with a high risk of infection, recurrence leading to limb amputation, and even death. Over half of DFUs are infected[6]; the incidence of DFU recurrence is 40% within 1 year and 65% within 3 years[3]. Despite various treatment strategies, approximately 20% of DFU patients with moderate and severe infections experience different levels of amputation[7,8]. According to a database analysis from the United Kingdom, the risk of death at 5 years for DFU patients was 2.5-fold greater than that for DM patients without DFU[9]. Additionally, treatment of DFU is costly, with nearly one-third of the estimated expenses for DM spent on DFU[10-12].

The great hazards of DFU necessitate a comprehensive understanding of its pathogenesis, aiming at increasing the cure rate, and decreasing the risks of infection, recurrence, and death. The progression of DFU is complex, with diabetic neuropathy (DN) and peripheral artery disease being the primary causes [13]. Multiple factors participate in the development of DFU; however, most previous studies have focused on environmental and controllable host factors. Recently, growing evidence has revealed that as a representative of host factors, single-nucleotide variations (SNVs) or single nucleotide polymorphisms are also involved in the development of DFU. This narrative review summarized current investigations regarding the roles of SNVs in the occurrence of DFU, thus providing new insights into the pathogenesis of DFU.

GENETIC SNVS INVOLVED IN DFU DEVELOPMENT

C-reactive protein

As an acute-phase response protein, C-reactive protein (CRP) levels increase in cases of tissue injury, infection, inflammation, and cancer[14,15]. Furthermore, it can be up to 1000 times the normal value in severe situations. A recent meta-analysis[16] indicated that the role of CRP is a promising biomarker for DFU infection evaluation. The CRP protein, encoded by the *CRP* gene, is located on chromosome 1q21-q23 and is 2.3 kb long[17]. Recent studies have reported that *CRP* genetic SNVs associated with the risk of developing DFU, including rs11265260, rs1800947, rs2794520, rs1130864, and rs3093059 (Table 1).

In a 2020 case-control study, Wang *et al*[17] investigated the potential influence of CRP SNVs, together with environmental factors, on the development of diabetic foot osteomyelitis (DFO) and prognosis of the patients with DFO. Altogether, 681 patients with DFO, 1053 patients without DFO, and 1261 healthy controls were included; and 11 CRP SNVs were analyzed. The results showed that rs11265260 (allele G), rs1800947 (allele G), rs2794520 (allele T), and rs1130864 (allele T) were linked to an increased risk to develop DFO in this Chinese cohort. Additionally, rs3093059 (allele C) showed a decreased risk. Furthermore, rs11265260 (allele G), rs1800947 (allele G), rs3093068 (allele G), and rs1130864 (allele T) were significant predictors of poor prognosis in these patients. Moreover, the GG and AG genotypes of rs11265260, the CG and GG genotypes of rs1800947, the TT genotype of rs3093059, and the CT and TT genotypes of rs113084 amplified the influences of smoking, alcohol consumption, cacosmia, and ulceration on progression from non-DFO to DFO. These outcomes imply that both

Table 1 Single nucleotide variations involving in the development of diabetic foot and its related complications

Ref.	Population or ethnicity	Total sample size (DF *vs* T2DM without DF) *vs* controls	Genes	SNVs reported	Potential influences of the SNVs on DF and DF related complications	Genotypes as risk or protective factors
Wang *et al*[17], 2020	Chinese	2995 (681 *vs* 1053 *vs* 1261)	*CRP*	rs11265260	Risk factor of DFO	GG + AG/GG
			CRP	rs1800947	Risk factor of DFO	GG + CG
			CRP	rs2794520	Risk factor of DFO	TT + CT/TT
			CRP	rs1130864	Risk factor of DFO	TT + CT/TT
			CRP	rs3093059	Protective factor against DFO	CC+CT/CC
Dhamodharan *et al*[23], 2015	Indian	515 (270[1] *vs* 139 *vs* 106)	*IL-6*	rs1800795	Protective factor against T2DM but not against DFU-DN	GC, CC
Erdogan *et al*[24], 2017	Turkish	204 (50 *vs* 35 *vs* 119)	*IL-6*	rs1800795	Risk factor of T2DM but not DFU	GG
Viswanathan *et al*[25], 2018	Indian	270 (without controls)	*IL-6*	rs1800795	Risk factor of severe wound infections	GC + CC
Dhamodharan *et al*[23], 2015	Indian	515 (270[1] *vs* 139 *vs* 106)	*TNF-α*	rs1800629	Risk factor of both T2DM and DFU-DN	GA, AA
Viswanathan *et al*[25], 2018	Indian	270 (without controls)	*TNF-α*	rs1800629	Risk factors of severe wound infections, ulcer grade of DF	GA + AA
				rs361525	Risk factor of ulcer grade of DF	GA + AA
Dhamodharan *et al*[23], 2015	Indian	515 (270[1] *vs* 139 *vs* 106)	*SDF-1*	rs1801157	Protective factor against T2DM and/or DFU-DN	GA, AA: T2DM; AA: DFU-DN
Viswanathan *et al*[25], 2018	Indian	270 (without controls)	*SDF-1*	rs1801157	Risk factors of severe wound infections and major amputations (foot/leg)	GA + AA
Amoli *et al*[34], 2011	Iranian	586 (247 *vs* 241 *vs* 98)	*VEGF*	rs699947	Protective factor against DFU	AA
Li *et al*[35], 2018	Chinese	288 (97 *vs* 88 *vs* 103)	*VEGF*	rs699947	Protective factor against DFU	AC, AA
Li[36], 2018	Chinese	229 (121 *vs* 108) (without healthy controls)	*VEGF*	rs2010963	Protective factor against DFU	CC
Teena *et al*[42], 2020	Indian	400 (100 *vs* 150 *vs* 150)	*NRF2*	rs35652124	Risk factors of DFU	TT
Teena *et al*[43], 2021	Indian	400 (100 *vs* 150 *vs* 150)	*NRF2*	rs182428269	Protective factor against T2DM and DFU	CC, CT
					Risk factor of T2DM and DFU	TT
Peng *et al*[45], 2018	Chinese	438 (142 *vs* 148 *vs* 148)	*SIRT1*	rs12778366	Protective factor against T2DM and DF	Allele C carriers
Cao *et al*[48], 2020	Chinese	430 (128 *vs* 147 *vs* 155)	*ICAM1*	rs5498	Protective factor against T2DM and DF	GG
			ICAM1	rs3093030	Protective factor against DF	CT + TT
Li[36], 2018	Chinese	229 (121 *vs* 108) (without healthy controls)	*MCP-1*	rs1024611	Risk factor of DFU	GG
Su *et al*[51], 2018	Chinese	400 (116 *vs* 135 *vs* 149)	*MCP-1*	rs1024611	Risk factor of DFU	AG, GG
Sadati *et al*[53], 2018	Iranian	257 (123 *vs* 134)	*eNOS*	eNOS Glu298Asp	Protective factor against	TT

		(without healthy controls)			DFU	
Erdogan *et al*[37], 2018	Turkish	182 (50 *vs* 57 *vs* 75)	*eNOS*	eNOS G894T	Risk factor of T2DM but not DFU	Not related to DFU onset
Zubair and Ahmad [58], 2018	Arabian	150 (50 *vs* 50 *vs* 50)	*HSP-70*	rs2227956	Risk factor of DFU	TT
					Protective factor of DFU	CC
Pichu *et al*[60], 2015	Indian	224 (79 *vs* 79 *vs* 66)	*HIF-1α*	rs11549465	Risk factor of DFU but not T2DM	CT
Pichu *et al*[61], 2018	Indian	529 (199 *vs* 185 *vs* 145)	*HIF-1α*	rs11549467	Risk factors of T2DM and DFU	GA
Pichu *et al*[65], 2017	Indian	906 (301 *vs* 305 *vs* 300)	*LOX*	rs1800449	Risk factor of DFU but not T2DM	AA
Mrozikiewicz-Rakowska *et al*[66], 2017	Polish	670 (204 *vs* 299 *vs* 167)	*ITLN1*	rs2274907	Risk factor of DF but not T2DM	TT
Meng *et al*[68], 2017	Scottish	3394 (699 *vs* 2695)	*MAPK14*	rs80028505	Risk factor of DFU	Not reported
Wifi *et al*[71], 2017	Egyptian	90 (30 *vs* 30 *vs* 30)	*TLRs*	rs5743836	Risk factor of DFU among T2DM patients	CT
Singh *et al*[70], 2013	Indian	255 (125 *vs* 130) (DFU *vs* healthy controls)	*TLRs*	rs4986790	Risk factor of DFU	AG/GG + AG
			TLRs	rs4986791	Risk factor of DFU	TT/CT/CT + TT
			TLRs	rs11536858	Risk factor of DFU	GG/AG/GG + AG
			TLRs	rs1927914	Risk factor of DFU	CC
			TLRs	rs1927911	Risk factor of DFU	CT/CT + TT
Nehring *et al*[72], 2013	Polish	877 (122 *vs* 293 *vs* 462)	*OPG*	rs2073617	Protective factor against DF among female patients	AG
			OPG	rs2073618	Risk factor of DF among T2DM patients	CC
Soroush *et al*[76], 2017	Iranian	212 (105 *vs* 107) (without healthy controls)	*VDR*	rs2228570	Risk factor of DFU among T2DM patients	TT + CT
Zhao *et al*[78], 2015	Chinese	300 (123 *vs* 97 *vs* 80)	*FIB*	rs6056	Risk factor of DF	CT, TT

[1]This group of 270 patients included 191 patients with DFU-DN and 79 patients with DFU-peripheral vascular disease.

DF: Diabetic foot; T2DM: Type 2 diabetes mellitus; SNVs: Single Nucleotide Variations; DFO: Diabetic foot osteomyelitis; DFU-DN: Diabetic foot ulcer with diabetic neuropathy; CRP: C-reactive protein; IL-6: Interleukin-6; TNF-α: Tumor Necrosis Factor-Alpha; SDF-1: Stromal cell Derived Factor-1; VEGF: Vascular Endothelial Growth Factor; NRF2: Nuclear Factor Erythroid-2-related Factor 2; SIRT1: Sirtuin 1; ICAM1: Intercellular Adhesion Molecule 1; MCP-1: Monocyte Chemoattractant Protein-1; eNOS: Endothelial Nitric Oxide Synthase; HSP-70: Heat Shock Protein-70; HIF-1α: Hypoxia inducible factor 1 alpha; LOX: Lysyl Oxidase; ITLN1: Intelectin 1 (Omentin); MAPK14: Mitogen-activated Protein Kinase 14; TLRs: Toll-Like receptors; OPG: Osteoprotegerin; VDR: Vitamin D receptor; FIB: Fibrinogen.

extrinsic and intrinsic factors participate in DFO pathogenesis, which may also affect patient prognosis. However, considering that this was a single-center study with a limited number of participants, future multicenter studies with larger sample sizes are necessary. Additionally, the potential effects of SNVs on plasma CRP levels still remain unclear. Previous studies have reported that several CRP SNVs such as rs1800947[18], rs1205[18,19], rs3091244[20] and rs3093059[21] might play a role in the development of diseases, partially *via* their influences on plasma CRP levels. Whether CRP SNVs influence CRP levels in patients with DFU requires further investigation.

Interleukin-6

Interleukin-6 (IL-6) is an important anti-inflammatory cytokine involved in the pathogenesis of type 2 diabetes mellitus (T2DM). Dysregulations of IL-6 and IL-6 signaling have been implicated in the etiology of autoimmune and inflammatory diseases, including T2DM[22]. One of the most frequently analyzed SNV sites is rs1800795; however, there is still a dispute regarding its role in the development of DFU (Table 1).

In 2015, Dhamodharan *et al*[23] reported a potential relationship between rs1800795 and susceptibility to DFU in an Indian population. The results revealed that the allele C of rs1800795 conferred significant protection against T2DM, but not against DFU. Similar outcomes were found in a Turkish population in

a study conducted by Erdogan *et al*[24]. It was observed that the G allele of rs1800795 is a risk factor for T2DM but not an independent risk factor for DFU. In 2018, Viswanathan *et al*[25] reported that compared with genotype GG, the mutant genotypes CC and CG of rs1800795 were linked to an elevated susceptibility to *Staphylococcus sp*., *Proteus morganii*, and *Citrobacter diversus* related infections in DFU patients. This finding suggests a potential role of such an SNV in specific microbial infections. In addition, they also observed that patients with GC and CC genotypes had significantly lower IL-6 levels than those with GG genotype. This finding implies that such an SNV participates in the occurrence of severe wound infections among DFU patients, partly *via* its influence on serological IL-6 levels. A recent meta-analysis[26] focused on the potential relationship between rs1800795 and the risk of developing microvascular complications in T2DM patients. Based on a pooled analysis of 14 eligible studies, the authors concluded that rs1800795 was unrelated to susceptibility to microvascular complications of T2DM. As in this study[26], all relevant microvascular complications (diabetic nephropathy, retinopathy, and foot disease) and multiple ethnicities were included, these parameters were synthesized as a whole entity for analysis, both of which may lead to high heterogeneity, and thus, a high risk of bias to the outcomes.

Tumor necrosis factor-alpha

As part of the humoral immunity against infections, tumor necrosis factor (TNF) is involved in inflammatory responses and plays an important role in the pathogenesis of multiple infectious diseases. As one of the most prominent members of the TNF cytokine family, TNF-α is primarily secreted by macrophages, natural killer cells, lymphocytes, and neurons. Recently, increasing evidence has revealed that TNF-α SNVs are associated with the development of various inflammatory disorders, such as chronic osteomyelitis[27], coronavirus disease 2019[28], and severe sepsis[29]. Recent studies have also found that TNF-α SNVs (primarily rs1800629 and rs361525) are linked to the development of DFU (Table 1).

In a 2015 study, in addition to the *IL-6* genetic SNV, Dhamodharan *et al*[23] and colleagues also noted that TNF-α SNVs rs1800629, but not rs361525, contributed to an increased risk of developing both T2DM and DFU-DN. In 2018, this group[25] also found that both rs1800629 and rs361525 were associated with severe microbial infections. Specifically, the genotypes GA and AA of rs1800629 displayed an elevated susceptibility to *Staphylococcus sp*.-, *Proteus morganii*-, and *Citrobacter diversus*-related infections. Genotypes GA and AA of rs361525 displayed an increased risk of developing *Proteus morganii*- and *Enterococcus sp*.- associated infections. In addition, rs1800629 and rs361525 were strongly correlated with ulcer grades. The potential influence of SNV genotypes on serological levels of inflammatory biomarkers was also examined. The authors noted that patients with GA and AA genotypes of rs1800629 had significantly lower levels of TNF-α and hsCRP than those with GG genotype[25]. Nonetheless, considering that the results were derived from two studies focusing on only one Indian population and by the same study group, future studies with different populations or ethnicities are warranted.

Stromal cell-derived factor-1

Stromal cell-derived factor-1 (SDF-1) is primarily responsible for homing and migration of endothelial progenitor cells and bone marrow-derived mesenchymal stem cells. It also plays a vital role in neovascularization[30]. Considering the pathophysiological changes in DFU, a potential role for SDF-1 is probable, and it is speculated that *SDF-1* genetic SNVs may be linked to the development of DFU (Table 1).

The outcomes of a 2015 study[23] demonstrated that the allele A of SDF-1 SNV rs1801157 conferred protection against T2DM and DFU. Specifically, compared with the normal glucose tolerance (NGT) group, frequencies of the GA and AA genotypes were significantly lower in both T2DM and DFU-DN groups. In addition, the frequency of the AA genotype was significantly lower in the DFU-DN group than that in the NGT group. Multiple logistic regression analysis revealed that both genotypes displayed significant protection against T2DM. While the AA genotype alone had a protective effect against DFU-DN. Moreover, the mean glycated hemoglobin level of the AA genotype was the lowest among the three genotypes, with the highest high density lipoprotein (HDL) cholesterol level. This finding can help explain the protective effect of rs1801157 may be achieved partly *via* its influences on glycated hemoglobin and HDL-cholesterol. In a subsequent 2018 study[25], the mutant genotypes GA and AA of such an SNV site were found to be associated with an elevated risk of developing *Staphylococcus sp*.- and *Enterococcus sp*.-related infections. Additionally, this SNV was correlated with an elevated risk of major amputation, even after adjusting for confounding factors. Whether the limb can be preserved among DFU patients depends on multiple factors aside from SNVs. Thus, caution should be taken exercised in this conclusion. However, in this study[25], the authors failed to find any positive influence of SDF-1 SNV on the serum levels of the biomarkers analyzed.

Vascular endothelial growth factor

As a mitogen in vascular endothelial cells[31], vascular endothelial growth factor (VEGF) can induce collagenases and contribute to angiogenesis by clearing the matrix. This facilitates the migration and sprouting of endothelial cells[32]. VEGF regulates transforming growth factor-β and platelet-derived

growth factor during the wound healing in patients with DFU[33]. Recent studies have reported positive relationships between *VEGF* genetic SNVs and susceptibility to DFU in different populations (Table 1).

In 2011, Amoli *et al*[34] examined the potential relationship between VEGF SNVs rs25648 and rs699947, and susceptibility to DFU in an Iranian population. The results revealed that the frequency of the AA genotype of rs699947 was significantly lower in patients with DFU than in patients with diabetes without DFU. Additionally, the frequency of allele A was lower than that in the controls. These results propose that rs699947 may be a protective factor against DFU, with allele A and AA genotypes acting as protective factors. In 2018, Li *et al*[35] analyzed the potential role of VEGF SNVs rs699947 and rs13207351 in the pathogenesis of DFU in a Chinese Han cohort. They also found that allele A of rs699947 was distinctly correlated with a decreased DFU risk, with AC and AA acting as protective genotypes. However, no statistical differences were noted between rs13207351 and susceptibility to DFU in this Chinese cohort. In the same year, the same study team[36] analyzed the potential link between VEGF SNV rs2010963 and the risk of developing DFU. Specifically, the frequencies of the CC genotype and allele C of rs2010963 were lower among patients with DFU than among those with T2DM without DFU. This observation demonstrates the protective role of this particular SNV against DFU. In addition, patients with DFU with the CC genotype had significantly higher VEGF levels than those with the GG genotype. Thus, the protective effect of rs2010936 against DFU may be exerted partly *via* its influence on serological VEGF levels. In another 2018 study, Erdogan *et al*[37] analyzed the association between VEGF SNV rs3025039 and the risk of DFU development in a Turkish population. However, no significant associations were identified with either the risk of DFU development or susceptibility to T2DM. Considering the limited sample size of this study (50 DFU patients and 57 diabetic patients without DFU), the results should be interpreted with caution. Future studies with larger sample sizes are necessary.

Nuclear factor erythroid-2-related factor 2

Among diabetic patients, prolonged hyperglycemia, and oxidative stress lead to the generation of excessive reactive oxygen species (ROS). These factors contribute to endothelial dysfunction, vascular damage, and delayed wound healing[38]. In hyperglycemia, ROS levels are higher than the intrinsic antioxidant capacity. This leads to subsequent alterations in the extracellular matrix and delayed wound healing[39]. As a transcription factor, nuclear factor erythroid-2-related factor 2 (NRF2) can maintain cellular redox homeostasis and transcribe the antioxidant response element to offer endogenous protection to cells by combating ROS. Post-translational modifications of SNVs profoundly associated with diabetes have been investigated. SNVs in the regulatory motifs of the *NRF2* gene can affect its binding capacity and, thus, inhibit the transcription[40]. Epidemiological and genetic studies have indicated that NRF2 promoter SNVs in diseases are linked to oxidative stress. This indicates that NRF2 polymorphisms are genetically predisposed to disease susceptibility[41].

In a 2020 cross-sectional study conducted in an Indian population, Teena *et al*[42] examined the potential link between the NRF2 SNV rs35652124 and susceptibility to DFU. Results based on 400 participants demonstrated that the frequency of the TT genotype among the DFU patients (52%) was significantly higher than that among T2DM patients without DFU (23%) and NGT controls (12%). These observations suggest that the TT genotype might be associated with an increased risk of DFU development in both T2DM patients and healthy controls. In addition, compared with the wild CC genotype, patients with DFU with the TT genotype expressed significantly increased TNF-α and IL-6 levels but a significantly decreased IL-10 level. Increases in TNF-α and IL-6 and a decrease in IL-10 levels have been reported to slow the chronic wound healing process, especially under insulin resistance [42]. Therefore, one underlying mechanism by which NRF2 SNV rs35652124 participate in the development of DFU is through dysregulation of key genes involved in redox homeostasis and wound healing. In 2021, the same group[43] assessed the role of rs182428269 in the development of DFU in the same population. Similarly, they found that the frequency of the TT genotype of DFU subjects was the highest among the three groups (DFU patients *vs* T2DM patients without DFU *vs* NGT controls = 42% *vs* 20% *vs* 11.4%). These findings demonstrates that rs182428269 is linked to an increased susceptibility to DFU occurrence, with the TT genotype as a risk factor. Additionally, compared with the CC and CT genotypes, the expression of NRF2 was significantly decreased among the DFU subjects with the TT genotype. Thus, one potential mechanism of SNV in the development of DFU is that they may affect the expression of NRF2. Based on the outcomes of the two NRF2 SNVs studies discussed, it is speculated that dysfunction of NRF2 by SNVs might be helpful in discerning disease development and progression in T2DM.

Sirtuin 1

Sirtuin 1 (SIRT1), also known as NAD-dependent deacetylase sirtuin-1, is downregulated in patients with T2DM and is associated with oxidative stress[44]. Previous studies have indicated that SIRT1 SNVs might alter their expressions or functions and thus contribute to the development of different disorders, such as neural or vascular lesions. Recent studies have shown that SIRT1 SNVs are also involved in DFU development (Table 1).

In a 2018 case-control study, Peng *et al*[45] explored the influence of SIRT1 SNVs (rs12778366 and rs3758391) on DF susceptibility and severity in T2DM patients. Based on the outcomes of 142 DF patients, 148 T2DM patients without DF, and 148 healthy controls, they noted that the C allele of rs12778366 was correlated with reduced DF susceptibility compared to the healthy controls and T2DM patients. This study demonstrates that the allele C of rs12778366 might act as a protective factor against DF onset. Moreover, the authors noted that the DF patients displayed significant downregulation of SIRT1 expression compared to those of the T2DM patients and the healthy controls. However, no statistical differences were identified regarding SIRT1 expression among different genotypes of rs12778366. Therefore, the detailed mechanisms of SIRT1 SNVs in the pathogenesis of DF and T2DM require further investigation.

Intercellular adhesion molecule 1

Intercellular adhesion molecule 1 (ICAM1) is an important regulator of cardiovascular disorders and peripheral neuropathy in patients with diabetes[46]. It is a cell surface glycoprotein expressed in immune and endothelial cells[47]. ICAM1 is regulated by the *ICAM1* gene located at 19p13.2; its SNVs in exon regions may influence the protein expression or function. Recent studies have indicated that *ICAM1* genetic SNVs participate in DF development (Table 1).

In a 2020 study[48] comprising 128 DF patients, 147 T2DM patients, and 155 healthy controls, Cao *et al*[48] examined the potential correlations between ICAM1 SNVs rs5498 and rs3093030, and susceptibility toward DF. The results revealed that the GG genotype of rs5498 was distinctly correlated with a decreased risk of developing both T2DM and DF, with the mutant allele G acting as a protective factor. In addition, the authors analyzed the effects of ICAM1 SNVs on DF characteristics. Notably, they observed that DF patients with the GG genotype had a significantly higher levels of serum creatinine than those with the AA genotype. However, the potential reasons remain unclear. In addition to rs5498, they also reported that individuals with the rs3093030 allele T had a reduced susceptibility to DF. Thus, rs3093030 may also act as a protective factor against the onset of DF. As this study only compared outcomes from clinical data, further studies should be performed to investigate the detailed protective mechanisms.

Monocyte chemoattractant protein-1

Monocyte chemoattractant protein-1 (MCP-1), also known as chemokine (C-C motif) ligand 2, is a potent cytokine that activates monocytes, macrophages, and lymphocytes[49]. Abnormal expression of MCP-1 may contribute to complications related to angiogenesis and vascular functions in T2DM patients[50]. Recently, growing evidence has shown that *MCP-1* genetic SNVs may be linked to DFU occurrence (Table 1).

In the aforementioned 2018 study, apart from *VEGF* SNV rs2010963, Li[36] reported the potential role of *MCP-1* SNV rs1024611 in the development of DFU. The results revealed that, compared with T2DM patients, the frequencies of both the G allele and GG genotype were increased among DFU patients. These findings implied that such a variant might be a risk factor for DFU onset among patients with T2DM. Additionally, the expression level of MCP-1 in patients with DFU with the GG genotype was significantly higher than those with the AA genotype. In the same year, Su *et al*[51] reported the potential influence of rs1024611 on the development of DFU in another Chinese cohort. Similarly, they also found that the G allele was associated with an increased risk of DFU development. Furthermore, individuals with the AG and GG genotypes had a higher risk of developing DFU. Similar findings were also obtained in that the GG genotype of rs1024611 was correlated with enhanced MCP-1 expression. This is consistent with previous findings by Li[36] that demonstrated that *MCP-1* genetic SNV rs1024611 may exert its biological effects partially *via* its influence on peripheral MCP-1 expression level. Moreover, Su *et al*[51] also found that the GG genotype of rs1024611 was correlated with a significantly higher epidermal thickness. Additionally, a significantly lower dermal thickness among patients with DFU was noted compared to those of AA and AG genotypes. This reveals another potential mechanism of such an SNV in DFU occurrence.

Endothelial nitric oxide synthase

As a key cellular signaling molecule, nitric oxide (NO) is an effective vasodilator that leads to smooth muscle relaxation. NO triggers oxidative stress by increasing free radicals and plays an important role in the pathogenesis of microvascular complications related to diabetes[52]. NO is produced through the oxidation of l-arginine by nitric oxide synthase (NOS); endothelial nitric oxide synthase (eNOS) is one of the three NOS isoforms (NOS3). Several eNOS SNVs have been linked to the occurrence of different types of disorders, including DFU (Table 1).

In a 2018 study, Sadati *et al*[53] examined associations between eNOS SNV *Glu298Asp* and the risk of DFU development in an Iranian cohort. Outcomes derived from 123 patients with DFU and 134 patients with T2DM without DFU revealed that the frequency of allele T was significantly lower in patients with DFU than in T2DM controls, with TT displaying a lower frequency in patients with DFU. This implies that the T allele may be protective against DFU. The authors explored levels of ROS and the total antioxidant power of plasma among patients with different genotypes. However, no significant

relationships were observed between such an SNV and levels of the two indicators. In another study carried out in a Turkish population, Erdogan *et al*[37] analyzed the potential effect of the eNOS SNV *G894T* on DFU susceptibility. The results revealed that the *G894T* allele T was a risk factor for diabetes but not a risk factor for DFU. As mentioned previously, considering the limited sample size of this study, future studies with more participants should be conducted.

Heat shock protein-70

Heat shock protein (HSP)-70 protein responds to stress and wound repair. Previous experiments[54,55] have shown significantly delayed or attenuated responses of cutaneous wound-induced HSP-70 expression in diabetic animals. It also functions as a key molecule in pathways linked to inflammation. Meanwhile, excessive production of inflammatory cytokines has been implicated in the pathogenesis of DFU[56]. A recent study of 946 subjects indicated that *HSP-70* genetic SNVs were strongly associated with renal complications in patients with T2DM in a South Indian population, demonstrating its possible role in T2DM and related complications.

Regarding the potential relationships between HSP-70 SNVs and DFU, a study[57] reported that HSP-70 SNVs were associated with the severity of DFU and surgical treatment outcomes. In 2018, Zubair and Ahmad[58] analyzed the potential role of HSP-70 SNV rs2227956 in the development of DFU in an Indian population. The results showed that a relatively higher frequency of the T allele was found among patients with DFU (7.3%) than among patients with T2DM (5.5%) and healthy controls (3.9%). The frequency of the TT genotype among patients with DFU was the highest (DFU *vs* T2DM *vs* healthy controls = 76% *vs* 44% *vs* 14%); and the frequency of the CC genotype among patients with DFU was the lowest (DFU *vs* T2DM *vs* healthy controls = 10% *vs* 30% *vs* 36%) among the three groups. This implies that the TT genotype may be a risk factor, whereas the CC genotype may be protective against DFU onset. Considering that only 150 participants were included (50 participants in each group), caution should be exercised in interpreting the findings.

Hypoxia inducible factor 1 alpha

Hypoxia inducible factor 1 alpha (HIF-1α) is considered a leading cause of various chronic diseases, including diabetes. It is a key regulator of genes involved in cellular response to hypoxia[59]. Growing evidence has shown that *HIF-1α* gene SNVs may be related to the development of DFU (Table 1).

In a 2015 study, Pichu *et al*[60] analyzed the potential link between HIF-1α SNV rs11549465 and the risk of developing DFU in an Indian population. The results confirmed that the frequencies of the CT genotype in both patients with T2DM and patients with DFU were higher than those in healthy controls. However, a significant difference was only found among the patients with DFU. This suggests that the CT genotype might be a risk factor for DFU but not for T2DM. The outcomes of subsequent analyses demonstrated that HIF-1α expression in patients with DFU was lower than that in patients with T2DM and healthy controls. In addition, patients with DFU with the CT genotype had a lower expression level of HIF-1α than those with the CC genotype. This observation implied that reduced HIF-1α expression might be associated with the development of DFU. In 2018, the same study[61] examined the role of HIF-1α SNV rs11549467 in DFU occurrence. The frequencies of the GA genotype were significantly higher in patients with T2DM and DFU than in healthy controls. Thus, this genotype was considered a risk factor for both T2DM and DFU onset. Similar to their previous study[60], a decreased expression level of HIF-1α was found among the patients with DFU compared to that in patients with T2DM and healthy controls. These findings suggest that HIF-1α may play an important role in DFU pathogenesis. However, in-depth mechanistic studies are required.

Lysyl oxidase

Lysyl oxidase (LOX), an extracellular matrix-modifying enzyme, is associated with cell proliferation, metastasis, angiogenesis, and wound healing. Elevated expression of the *LOX* gene and accompanying cross-linked collagen fibrils in diabetic skin may lead to changes in tissue mechanical properties. These features are important for the regulation of tensile and elastic features of connective tissues[62,63]. LOX expression may be positively regulated by high glucose levels in diabetic skin[64]. LOX SNVs have also been associated with DFU development (Table 1).

In a 2017 case-control study, Pichu *et al*[65] analyzed the potential relationship between LOX SNV rs1800449 and susceptibility to DFU in an Indian population. The outcomes of 906 participants showed a significantly higher frequency of allele A among the DFU patients (42 %) than that among the controls (33%), with the AA genotype as a risk factor for DFU. Moreover, the LOX transcript level linked to the AA genotype among patients with DFU was significantly higher than that of the AA genotype among patients with T2DM and controls. This suggests that the increased expression of LOX may participate in the onset of DFU.

Intelectin 1

Intelectin 1 (ITLN1), also known as omentin, is encoded by the *ITLN1* gene located on the long arm of chromosome 1 (1q21.3)[66]. Mrozikiewicz-Rakowska *et al*[66] examined the potential role of rs2274907 in the development of DFU in a Polish population. Based on 670 individuals, they found that the T allele

was more frequent in the DF group than in the control group. Therefore, the TT genotype is a possible risk factor. In addition, this effect was sex-specific and observed in males (Table 1). Although the influence of such an SNV on the concentration of omentin in the DFU patients remains unclear, the authors introduced the underlying mechanisms regarding the protective effects of omentin on endothelium and smooth muscle cells for detail[66]. Omentin is able to stimulate NO production, leading to the endothelium–dependent vasodilation. In addition, omentin can also suppress the inflammatory response in endothelial cells by inhibiting the c-Jun N-terminal kinase activation *via* the AMP-activated protein kinase/eNOS signaling pathway. Furthermore, omentin decreases the adhesion of monocytes to endothelial cells by reducing expression of vascular cell adhesion protein-1 on the surface of monocytes as well as reducing the expression of intercellular adhesion molecule-1. Aside from endothelium, omentin also displayed an inhibitory effect on TNF–α–induced adhesion of monocytes in vascular smooth muscle cells of the rat. Nonetheless, the detailed mechanisms of ITLN1 SNVs in the development of DFU are still largely unknown and requires further research.

Mitogen-activated protein kinase 14

Mitogen-activated protein kinase 14 (MAPK14) targets a broad range of nuclear and cytosolic substrates that participate in a wide variety of cellular processes, such as proliferation, differentiation, apoptosis, transcription regulation, and development. It is a kinase involved in cellular responses to extracellular stimuli, such as pro-inflammatory cytokines or physical stress[67]. In a 2017 study, Meng *et al*[68] analyzed potential SNVs related to the development of DFU in a Scottish population. The results showed that rs80028505 was associated with increased susceptibility to DFU in a Scottish cohort (Table 1).

Toll-like receptors

Toll-like receptors (TLRs) superfamily members play a fundamental role in detecting invading pathogens or damage and initiating the innate immune system. Aberrant activation of TLRs exaggerates T cell-mediated autoimmune activation, causing unwanted inflammation and promoting DFU[69]. Recent studies have indicated that TLR SNVs are involved in DFU development (Table 1).

In a 2013 study, Singh *et al*[70] reported potential associations between TLR4 SNVs (rs4986790, rs4986791, rs11536858, rs1927911, and rs1927914) and susceptibility to DFU in an Indian population study. The results showed that these TLR4 SNVs correlated with an increased risk of developing DFU. They also reported 15 haplotypes with a frequency greater than 1%, and outcomes revealed that the haplotype ACATC displayed a strong association with DFU risk. In contrast, the haplotypes ATATC and ATGTT were noted to be protective against DFU. Furthermore, the authors also introduced two different models to predict the risk of DFU development. They proposed that the artificial neural network model was better than the multivariate linear regression model. In 2017, Wifi *et al*[71] analyzed the relationship between *TLR2* (rs3804100) and *TLR9* (rs5743836) SNVs and the risk of developing DF in an Egyptian population. The results suggest that rs5743836, rather than rs3804100, is associated with an elevated risk of DFU development among patients with T2DM. However, considering the limited number of eligible participants, cautious attitudes should be taken towards inferring the outcomes and conclusions.

Osteoprotegerin

Osteoprotegerin (OPG) plays a key role in the regulation of bone resorption and it belongs to the TNF superfamily. In a 2013 study, Nehring *et al*[72] examined the links between three SNVs (rs2073617, rs2073618, and rs3134069) located in the *TNFRSF11B* gene and the risk of DF development in a Polish population. The results showed that the C allele and CC genotype of rs2073618 were risk factors for DF onset in T2DM patients. For rs2073617, the mutant allele A and AG genotypes were protective against DF (Table 1).

Vitamin D receptor

Growing evidence has demonstrated that vitamin D receptor (VDR) SNVs are involved in the pathogenesis of several inflammatory disorders, such as fracture-related infection[73], tuberculosis[74], and periodontitis[75]. In a 2017 study, Soroush *et al*[76] analyzed the role of VDR SNV rs2228570 in the development of DFU in an Iranian population. The results showed that the frequencies of genotypes TT and TC among patients with DFU were significantly higher than those without DFU. This finding implies that such genotypes of this SNV present a risk factor to this cohort. In addition, they also evaluated the expression levels of oxidative stress indicators, thiobarbituric acid reactive substances (TBARS), and ferric-reducing ability of plasma (FRAP) among different genotypes of the SNV. The results showed that the median level of TBARS among patients with the TT and TC genotypes was significantly higher than that of the CC genotype. However, no statistical difference in FRAP levels between the two groups was noted. Nonetheless, no significant relationships were found between the genotypes and TBARS or FRAP levels among healthy controls. This suggests that one underlying mechanism of VDR SNV rs2228570 in DFU pathogenesis is partly *via* its influence on TBARS levels (Table 1).

Fibrinogen

Fibrinogen (FIB) and fibrin play important roles in multiple biological processes, including fibrinolysis, blood clotting, inflammation, wound healing, cellular and matrix interactions, and neoplasia. A recent study[77] confirmed the definitive role of FIB as a promising inflammatory marker in the discrimination of DFU. In a 2015 study, Zhao *et al*[78] investigated the correlation between *FIB* SNV rs6056 polymorphism and susceptibility towards DF in a Chinese population. Outcomes based on 300 subjects demonstrated that the mutant allele T, CT, and TT genotypes were risk factors for DF onset, following univariate logistic regression analysis. The TT genotype was associated with a relatively higher serological FIB level (Table 1).

LIMITATIONS AND FUTURE PERSPECTIVES

Increasing evidence has suggested that, in addition to extrinsic factors, intrinsic factors such as SNVs also participate in the development of DFU. However, these investigations had limitations. First, the sample sizes of most studies were limited; therefore, caution should be exercised regarding inferring relevant outcomes and conclusions. Second, most of the studies were conducted in Asian countries (*e.g.*, India, China, and Iran). To comprehensively evaluate the potential roles of SNVs in the pathogenesis of DFU, investigations focusing on different populations or ethnicities should be conducted in the future. Third, as the majority of the analyzed studies only reported preliminary findings based on case-control comparison outcomes, there is still a lack of in-depth research on mechanisms.

Based on these limitations, future studies should focus on two primary aspects. On the one hand, multi-center studies with larger sample sizes and diverse populations should be conducted. This will ensure a more accurate and comprehensive assessment of the potential roles of SNVs in the development of DFU. On the other hand, the detailed mechanisms should be investigated from different perspectives for SNVs with clinical significance.

CONCLUSION

Based on recent findings, SNVs located in the genes of *CRP* (rs11265260, rs1800947, rs2794520, rs1130864, rs3093059), *IL-6* (rs1800795), *TNF-α* (rs1800629, rs361525), *SDF-1* (rs1801157), *VEGF* (rs699947, rs2010963), *NRF2* (rs35652124, rs182428269), *SITR1* (rs12778366), *ICAM1* (rs5498, rs3093030), *MCP-1* (rs1024611), *eNOS* (Glu298Asp), *HSP-70* (rs2227956), *HIF-1α* (rs11549465, rs11549467), *LOX* (rs1800449), *ITLN1* (rs2274907), *MAPK14* (rs80028505), *TLRs* (rs5743836, rs4986790, rs4986791, rs11536858, rs1927914), *OPG* (rs2073617, rs2073618), *VDR* (rs2228570), and *FIB* (rs6056) may be important molecular players influencing the development and progression of DFU.

FOOTNOTES

Author contributions: Hu YJ and Song CS contributed equally to this study; Hu YJ and Jiang N conceived and designed the study; Song CS searched the literature; Song CS and Jiang N drafted the article; Hu YJ, Song CS, and Jiang N revised the manuscript; All authors approved the final version of the submitted article.

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ORIGINAL ARTICLE

Observational Study

Baseline moderate-range albuminuria is associated with protection against severe COVID-19 pneumonia

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Abstract

BACKGROUND

Diabetes mellitus is considered a leading contributor to severe coronavirus disease 2019 (COVID-19).

AIM

To characterize differences between hospitalized diabetic patients with *vs* without COVID-19, and parameters associated with COVID-19 severity for prediction.

METHODS

This case-control study included 209 patients with type 2 diabetic mellitus hospitalized at the Galilee Medical Center (Nahariya, Israel) and recruited between September 2020 and May 2021, 65 patients with COVID-19 infection in dedicated wards and 144 COVID-19-negative patients in internal medicine wards hospitalized due to other reasons. Clinical parameters - including age, type of antiglycemic medications, presence of retinopathy, smoking history, body mass index (BMI), glycosylated hemoglobin, maximum neutrophil:lymphocyte ratio

(NLR_{max}), C-reactive protein (CRP), estimated glomerular filtration rate (eGFR), and albumin (blood and urine) - were compared between the two primary patient groups, and then between COVID-19-negative patients hospitalized due to infectious *vs* non-infectious disease. Finally, we explored which parameters were associated with severe COVID-19 pneumonia.

RESULTS

COVID-19-negative patients were older (63.9 ± 9.9 *vs* 59.8 ± 9.2, $P = 0.005$), and had longer duration of diabetes ($P = 0.031$), lower eGFR ($P = 0.033$), higher albumin ($P = 0.026$), lower CRP ($P < 0.001$), greater smoking prevalence ($P < 0.001$), and more baseline albuminuria (54.9% *vs* 30.8%, $P = 0.005$) at admission; 70% of COVID-19 patients with albuminuria had moderate-range albuminuria (albumin:creatinine 30-300 mg/g). Most of the patients with albuminuria had chronic kidney disease stage II (CKD II). Oral antiglycemic therapies were not significantly different between the two groups. Multivariable logistic regression showed that higher BMI was significantly associated with severe COVID-19 (OR 1.24, 95%CI: 1.01-1.53, $P = 0.04$), as was higher NLR_{max} (OR 1.2, 95%CI: 1.06-1.37, $P = 0.005$). Surprisingly, pre-hospitalization albuminuria, mostly moderate-range, was associated with reduced risk (OR 0.09, 95%CI: 0.01-0.62, $P = 0.015$). Moderate-range albuminuria was not associated with bacterial infections.

CONCLUSION

Moderate-range albuminuria in COVID-19-positive diabetic patients with CKD II is associated with less severe COVID-19. Further studies should explore this potential biomarker for risk of COVID-19-related deterioration and early interventions.

Key Words: Diabetes mellitus; COVID-19; Albuminuria; Severity; Chronic kidney disease; Immunomodulation

Core Tip: Type 2 diabetes mellitus and its risk factors are considered to be contributors to severe coronavirus disease 2019 (COVID-19). In this study, we analyzed our single-center clinical data of adults with type 2 diabetes between September 2020 and May 2021 to determine the impact of risk factors on severity of COVID-19 pneumonia. Surprisingly, we found that moderate-range pre-hospitalization albuminuria was associated with reduced risk of severe COVID-19 pneumonia. Further studies are needed to explore this association and pathogenesis relating to immunomodulation, which may indicate a biomarker for patients at reduced risk for COVID-19-related deterioration that may translate to therapeutic interventions.

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INTRODUCTION

In December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the causative pathogen for coronavirus disease 2019 (COVID-19) pneumonia[1]. COVID-19 infection can cause various symptoms of varying severity, starting from mild disease with upper respiratory tract infection and continuing to moderate and severe pneumonia with a systemic inflammatory response syndrome, acute respiratory distress syndrome (ARDS), multi-organ involvement, and shock[2].

Several risk factors for severe COVID-19 disease have been described, and include advanced age, male sex, smoking history, and underlying chronic diseases such as cardiovascular disease (CVD), diabetes mellitus, obesity, underweight, and chronic kidney disease (CKD) [defined as estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m²], as well as socioeconomic deprivation[3,4]. The presence of diabetes and the individual degree of hyperglycemia appear to be independently associated with COVID-19 severity and increased mortality[5].

Given the compromised immune function in patients with diabetes, especially innate immunity with impaired natural killer (NK) cells[5-7], impaired T cell responses with increased Th1 and Th17 cells, reduced T regulatory cells, and altered cytokine response[8], diabetes is considered to be a risk factor for

severe COVID-19 pneumonia[5,9]. A recent retrospective study demonstrated elevated cytokines, imbalance of Th1/Th2 secreted cytokines and reduced levels of CD8+ T cells and NK cells in patients with diabetes suffering from COVID-19 pneumonia compared to patients without diabetes, with reduced level of CD8+ T cells and NK cells being more pronounced in non-survivors[10]. In addition, other risk factors for severe COVID-19 pneumonia are associated with diabetes, *e.g.*, obesity, hyperglycemia, CVD, and CKD[1-3].

In this case-control study, we aimed to characterize the differences between patients with diabetes hospitalized in internal medicine departments and patients with diabetes suffering from COVID-19 pneumonia in designated wards at the Galilee Medical Center. Among the patients with COVID-19 infection, we explored clinical parameters that were associated with severe COVID-19 pneumonia for predictive value.

MATERIALS AND METHODS

Participants

Data were analyzed from 209 type 2 diabetic patients hospitalized at the Galilee Medical Center between September 2020 and May 2021 and participating in a study evaluating the prevalence of and related factors for *de novo* positive COVID19 serology (ethical committee approval number 0073-21-NHR, dated 05-Jul-2021). Sixty-five patients suffering from COVID-19 infection were hospitalized in the COVID-19 wards and 144 patients with other diseases were hospitalized in the internal medicine wards, the latter recruited concurrently for unbiased comparison (Figure 1). Diabetes was defined by glycosylated hemoglobin (HbA1c) ≥ 6.5% and by medical history of type 2 diabetes diagnosis in the past. Patients hospitalized in the internal medicine wards were recruited during their hospitalization period, while patients suffering from COVID-19 pneumonia were recruited after their discharge from the hospital. All participants signed the informed consent form as approved by the Galilee Medical Center Helsinki Committee (investigational review board). The total number of participants was reached per enrollment criteria of patients hospitalized during the selected time period.

Design and procedures

This case-control study included the following two parts: (1) First we compared the clinical parameters between the two primary patient groups, followed by a comparison of the clinical characteristics between patients hospitalized due to infectious *vs* non-infectious disease in the internal medicine wards; and (2) Second, we explored which clinical parameters were associated with severe COVID-19 pneumonia.

Demographic, clinical, and laboratory parameters were collected from electronic hospital and community records using Chameleon and Ofek software, respectively. The following baseline parameters were recorded: Age, sex, religion, type of antiglycemic medications [metformin, dipeptidylpeptidase-4 (DPP-4) inhibitors, sulfonylurea, sodium glucose cotransporter-2 (SGLT2) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists, and insulin], presence of retinopathy, smoking history, body mass index (BMI), and HbA1c. The following parameters were collected during the hospitalization period: maximum neutrophil:lymphocyte ratio (NLR_{max}) and C-reactive protein (CRP) (plus values at admission), eGFR, and albumin. COVID-19 infection was defined as a positive SARS-CoV-2 polymerase chain reaction.

Baseline moderate-range albuminuria was defined as an albumin-to-creatinine ratio between > 30 and < 300 mg/g in two urine analyses performed during the 18 mo prior to hospitalization; macroalbuminuria was defined as > 300 mg/g. eGFR was calculated by using the CKD Epidemiology Collaboration creatinine equation[11]. Baseline HbA1c and eGFR were calculated as the average of up to two last values of the respective tests during the 12 mo prior to hospitalization. Retinopathy was defined according to fundoscopic examination conducted during the 18 mo prior to hospitalization (it included background retinopathy, proliferative retinopathy, or macular edema).

Quantitative variables other than albuminuria were not divided into subgroups. Albuminuria was divided dichotomously for patients with and without, based on known categorization by urine-albumin-to-creatinine ratio (< 30 mg/g and ≥ 30 mg/g); and for those with, further divided into three groups according to albuminuria severity (albuminuria < 30 mg/g, 30-300 mg/g, and > 300 mg/g).

The severity of COVID-19 infection was determined in accordance with the following Israel Ministry of Health criteria published July 12, 2020 (according to the United States National Institutes of Health): (1) Mild illness when there are symptoms of mild viral upper respiratory tract infection; (2) Moderate illness per imaging and oxygen saturation ≥ 94% on room air; (3) Severe illness when one of the following criteria were met: respiratory rate > 30 breaths/minute, oxygen saturation < 94% on room air, PaO_2/FiO_2 < 300 mmHg, or lung infiltration > 50%; and (4) Critical illness per hemodynamic instability, need for mechanical ventilation, and/or multiorgan failure.

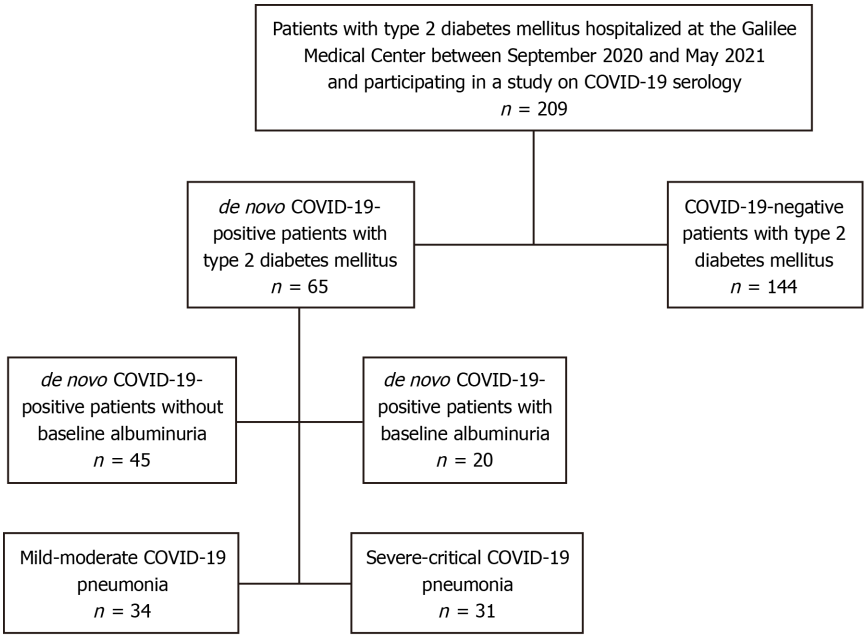

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Figure 1 Enrolled participants and analysis groups. COVID-19: Coronavirus disease 2019.

Statistical analysis

Quantitative variables were analyzed for mean (SD), median, and interquartile range (IQR). Categorical variables were analyzed with frequencies and percentages.

Differences between groups for continuous variables were compared using independent sample *t*-test or Mann-Whitney test. We chose independent sample *t*-test when the compared variables did not deviate significantly from the normal distribution. Differences between groups for categorical variables were compared with Chi-square or Fisher's exact tests (if expectancy < 5).

Correlations between continuous variables were examined with Spearman's correlation coefficient test, which was chosen over Pearson's correlation coefficient test according to the variable's distribution shape. Multivariable logistic regression modelling was used to determine the risk factors for severe or critical COVID-19 and separately for infectious compared to non-infectious disease in patients without COVID-19 infection. In the multivariable analysis, the severity of COVID-19 pneumonia and presence of infectious disease were the dependent variables, while the following baseline parameters were independent variables: age, sex, BMI, last eGFR measured before hospitalization, HbA1c, NLR_{max}, and albuminuria before hospitalization. These risk factors were chosen according to the univariable analysis results and theoretical considerations. We defined the following three models according to variables: Model 1 included sex, age, and BMI; Model 2 included Model 1 variables plus HbA1c, eGFR, and NLR_{max}; and Model 3 included Model 2 variables plus the presence of albuminuria. Model 3 presents an adjustment of background and clinical measures (*e.g.*, age, eGFR, NLR_{max}, *etc.*) for the albuminuria variable.

Odds ratios (OR) and 95% confidence intervals (CI) for OR are provided as estimates of risk for each variable.

Analyses were performed with IBM SPSS Statistics software version 27.0 (Chicago, IL, United States). A P value of < 0.05 was considered statistically significant. Two-sided P values are presented unless otherwise specified.

RESULTS

Between September 2020 and May 2021, 65 patients with diabetes suffering from COVID-19 infection and 144 diabetic COVID-19-negative patients hospitalized due to other reasons were enrolled in this study.

Clinical characteristics of patients with diabetes, with and without COVID-19 infection

Clinical characteristics of patients with diabetes, with COVID-19 infection compared to diabetic patients without COVID-19 infection, are presented in Table 1. Patients without COVID-19 were older than patients with COVID-19 (63.9 ± 9.9 years compared to 59.8 ± 9.2 years, respectively, P = 0.005), had lower prevalence of smoking (6.2% compared to 33.3%, P < 0.001), longer duration of diabetes (P =

Table 1 Clinical and demographic characteristics of patients with type 2 diabetes, with and without coronavirus disease 2019 infection

	Patients without COVID-19 infection, *n* = 144	Patients with COVID-19 infection, *n* = 65	*P* value
Age (yr), mean (SD)	63.9 (9.9)	59.8 (9.2)	0.005[1]
Median (IQR)	65 (57-71)	61 (53-66)	
Sex, female, *n* (%)	50 (34.7)	32 (49.2)	0.066[2]
Population group, *n* (%)			0.067[2]
Jews	63 (44.7)	20 (30.8)	
Arabs	78 (55.3)	45 (69.2)	
BMI (kg/m^2), mean (SD)	30.7 (5.9)	32.1 (4.6)	0.09[1]
Median (IQR)	30.3 (27.4-33.8)	31.6 (29.1-34.7)	
Diabetes duration (yr), median (IQR)	13 (8-17.8)	10 (5.5-14.5)	0.031[3]
eGFR (mL/min/1.73 m^2 body surface area) at baseline, median (IQR)	85.4 (62.2-97.6)	91.9 (75.3-101.0)	0.033[3]
HbA1c (%), median (IQR)	7.6 (6.5-9.1)	7.4 (6.6-9.1)	1.00[3]
Metformin, *n* (%)	110 (76.9)	56 (86.2)	0.14[2]
DPP-4 inhibitors, *n* (%)	29 (20.1)	18 (27.7)	0.28[2]
Sulfonylurea, *n* (%)	8 (5.6)	7 (10.8)	0.25[4]
SGLT2 inhibitors, *n* (%)	36 (25.0)	18 (27.7)	0.73[2]
GLP-1 agonists, *n* (%)	25 (17.4)	12 (18.5)	1.00[2]
Basal insulin, *n* (%)	60 (41.7)	18 (27.7)	0.064[2]
Prandial insulin, *n* (%)	30 (21.0)	6 (9.2)	0.047[2]
Current smoking, *n* (%)	48 (33.3)	4 (6.2)	< 0.001[2]
No albuminuria, *n* (%)	65 (45.1)	45 (69.2)	0.002[2]
Albuminuria < 30 mg/g, *n* (%)	79 (54.9)	20 (30.8)	0.005[3]
Albuminuria 30-300 mg/g, *n* (%)	65 (45.1)	14 (21.5)	
Albuminuria > 300 mg/g, *n* (%)	14 (9.7)	6 (9.2)	
Retinopathy, *n* (%)	21 (21.4)	7 (15.2)	0.50[2]
NLR_{max} at hospitalization, median (IQR)	4.0 (2.5-7.8)	6.5 (2.6-10.0)	0.12[3]
CRP (mg/L) at admission, median (IQR)	9.7 (4.8-45.4)	71.8 (12.2-145.9)	< 0.001[3]
CRP_{max} (mg/L) at hospitalization, median (IQR)	11.3 (5.2-68.1)	86.6 (12.2-167.6)	< 0.001[3]
eGFR (mL/min/1.73 m^2 body surface area) at hospitalization, median (IQR)	86.0(62.2-96.0)	92.1(76.2-100.8)	0.030[3]
Albumin, median (IQR)	3.8 (3.4-4.0)	3.6(3.2-3.9)	0.026[1]

[1]Independent sample *t*-test.
[2]Chi-square test.
[3]Mann-Whitney test.
[4]Fisher's exact test.
COVID-19: Coronavirus disease 2019; IQR: Interquartile range; BMI: Body mass index; HbA1c: Glycosylated hemoglobin; eGFR: Estimated glomerular filtration rate; DPP-4: Dipeptidyl-peptidase 4; SGLT2: Sodium/glucose cotransporter 2; GLP-1: Glucagon-like peptide 1; NLR_{max}: Maximum neutrophil:lymphocyte ratio; CRP: C-reactive protein; CRP_{max}: Maximum C-reactive protein.

0.031), lower eGFR (P = 0.033), higher albumin (P = 0.026), and lower CRP at admission, as well as lower maximum value of CRP (CRP_{max}) during hospitalization (P < 0.001). Interestingly, baseline albuminuria was more common in patients without COVID-19 infection (54.9% compared to 30.8%, P = 0.005). There was a trend toward a higher percentage of insulin therapy in patients without COVID-19 (P = 0.047 and P = 0.064 for prandial and basal insulin, respectively). Use of other antiglycemic therapies were not significantly different between the two groups.

Clinical characteristics of patients with diabetes suffering from COVID-19, with and without albuminuria

Among the 65 patients suffering from COVID-19 infection in this cohort, 20 had documented albuminuria prior to hospitalization, whereas 45 did not. Most of the patients with albuminuria had CKD II and moderately increased albuminuria (A2) (Table 2). Basal insulin therapy was more common in patients with albuminuria (50% compared to 17.8%, P = 0.015). As expected, patients with albuminuria had significantly higher values of HbA1c (median 8.9% and IQR_{25-75} 7.3%-10.4%, compared to 7.2% and 6.6%-8.4%, respectively, P = 0.02). Other parameters were not significantly different between the two groups (Table 2).

Predictors of severe COVID-19 pneumonia

Univariable analysis demonstrated increased risk for severe COVID-19 pneumonia with higher inflammatory markers NLR_{max} and CRP (P < 0.001) and lower albumin level (P < 0.001). Oral antiglycemic therapies were not significantly different between patients with moderate or severe pneumonia (Table 3).

The following variables were considered to be confounders due to putative correlation with albuminuria and COVID-19 severity: age, BMI, eGFR, HbA1c, and NLRmax. We therefore examined the correlation between each of these variables and albuminuria (see Table 2) and COVID-19 severity (see Table 3). According to the univariable analysis (Tables 2 and 3), HbA1c was found to be significant correlated with albuminuria (P = 0.02), but was not found to be significant in the COVID-19 severity univariable analysis (P = 0.93); NLR_{max} was not found to be correlated with albuminuria (P = 0.48), but was found to be correlated with COVID-19 severity (P < 0.001); age and BMI were found only to trend toward correlations with albuminuria (P = 0.16 and P = 0.19 respectively) and COVID-19 severity (P = 0.26, P = 0.22), possibly due to the small sample size; eGFR was not found to correlate with either albuminuria or COVID-19 severity (P = 0.90 and P = 0.78, respectively).

Because of the theoretical consideration and the above findings, we decided to include those variables in the multivariable analysis. Given the association between sex and COVID-19 severity reported in the literature, we included this variable in the univariable analysis, wherein male sex showed only a trend toward correlation with COVID-19 severity (P = 0.14), with no correlation for albuminuria (P = 0.60).

Variables associated with severe COVID-19 pneumonia in the multivariable logistic analysis according to the Models 1-3 are presented in Table 4, and the multivariable regression in Model 3 is depicted in Figure 2. The dependent variable is the severity of COVID-19 pneumonia, while the following parameters are independent variables: age, sex, BMI, last eGFR measured before hospitalization, HbA1c, NLR_{max}, and albuminuria before hospitalization. In the final model, as expected, a higher BMI was significantly associated with severe COVID-19 pneumonia (OR 1.24, 95%CI: 1.01-1.53, P = 0.04), as was higher NLR_{max} (OR 1.20, 95%CI: 1.06-1.37, P = 0.005). Surprisingly, the presence of moderate-range albuminuria before hospitalization was associated with reduced risk (OR 0.09, 95%CI: 0.01-0.62, P = 0.015). Of note, 70% of COVID-19 patients with proteinuria had moderate-range albuminuria.

As expected, moderate correlation strength was found between age and diabetes duration in the COVID-19 group (Spearman's correlation coefficient test r = 0.58, P < 0.001). Given this correlation, only age was included in the multivariable regression model. Similarly, moderate-to-strong correlation strength was found between NLR_{max} and CRP_{max} in the COVID-19-positive patients (Spearman's correlation coefficient test r = 0.59, P < 0.001 for the COVID-negative patients, and r = 0.73, P < 0.001 for the COVID-19-positive patients). Given the significant correlation between CRP and NLR_{max}, only NLR_{max} was included in the multivariable regression model.

Moderate range albuminuria was not associated with bacterial infections

Given the surprising protective association between moderate-range albuminuria and severe COVID-19 infection, we wanted to explore whether this association is similarly observed in bacterial infections. We hypothesized that this protective association is specific for viral infections such as COVID-19 and not to bacterial infections. For this, similar multivariable logistic regression models were conducted in COVID-19-negative patients without bacterial infections *vs* patients with bacterial infections to characterize which variables are associated with the latter. Variables associated with the absence *vs* presence of bacterial infection in the multivariable analysis according to the Models 1-3 are presented (Table 5). In the final model, NLR_{max} was significantly associated with bacterial infection. The protective effect of albuminuria was not observed with regard to bacterial infection. The apparent protective effect of moderate-range albuminuria in patients with CKD II was specific to COVID-19 infection in this cohort, but may be relevant to other viral infections as well.

DISCUSSION

Diabetes mellitus has been associated with severe COVID-19 pneumonia[3-5,8]. Hyperglycemia increases SARS-CoV-2 replication in human monocytes, and glycolysis sustains SARS-CoV-2 replication

Table 2 Clinical and demographic characteristics of patients type 2 diabetes and coronavirus disease 2019 infection, with and without albuminuria

	Diabetic patients without baseline albuminuria, *n* = 45	Diabetic patients with baseline albuminuria, *n* = 20	*P* value
Age (yr), mean (SD)	58.7 (9.2)	62.2 (9.0)	0.16[1]
Median (IQR)	60.0 (50.5-65.0)	63.0 (56.5-68.8)	
Sex, female, *n* (%)	21 (46.7)	11 (55.0)	0.60[2]
Population group, *n* (%)			0.77[2]
Jews	13 (28.9)	7 (35.0)	
Arabs	32 (71.1)	13 (65.0)	
BMI (kg/m^2), mean (SD)	31.6 (4.5)	33.2 (4.6)	0.19[1]
Median (IQR)	30.9 (28.6-33.7)	32.3 (29.7-35.9)	
Diabetes duration (yr), median (IQR)	10.0 (5.5-14.0)	11.0 (5.3-17.0)	0.25[1]
eGFR (mL/min/1.73 m^2 body surface area) at baseline, median (IQR)	90.5 (77.8-99.5)	95.3 (69.6-101.1)	0.90[3]
HbA1c (%), median (IQR)	7.2 (6.6-8.4)	8.9 (7.3-10.4)	0.02[3]
Metformin, *n* (%)	41 (91.1)	15 (75)	0.12[4]
DPP-4 inhibitors, *n* (%)	15 (33.3)	3 (15)	0.15[2]
Sulfonylurea, *n* (%)	5 (11.1)	2 (10)	1.00[4]
SGLT2 inhibitors, *n* (%)	15 (33.3)	3 (15)	0.15[2]
GLP-1 agonists, *n* (%)	8 (17.8)	4 (20)	1.00[4]
Basal insulin, *n* (%)	8 (17.8)	10 (50)	0.015[2]
Prandial insulin, *n* (%)	2 (4.4)	4 (20)	0.067[4]
Current smoking, *n* (%)	2 (4.4)	2 (10)	0.58[4]
Retinopathy, *n* (%)	4 (12.9)	3 (20)	0.67[4]
NLR_{max}, median (IQR)	6.2 (2.5-9.4)	6.7 (2.6-18.0)	0.48[3]
CRP (mg/L) at admission, median (IQR)	90.4 (10.9-153.6)	43.9 (12.5-106.8)	0.33[3]
CRP_{max} (mg/L) at hospitalization, median (IQR)	92.8 (11.4-171.3)	61.0 (12.5-157.3)	0.60[3]
eGFR (mL/min/1.73 m^2 body surface area) at hospitalization, median (IQR)	90.3 (77.0-98.4)	94.3 (67.8-101)	0.76[3]
Albumin, median (IQR)	3.6 (3.3-4.0)	3.6 (3.1-3.9)	0.25[1]

[1]Independent sample *t*-test.
[2]Chi-square test.
[3]Mann-Whitney test.
[4]Fisher's exact test.
IQR: Interquartile range; BMI: Body mass index; HbA1c: Glycosylated hemoglobin; eGFR: Estimated glomerular filtration rate; DPP-4: Dipeptidyl-peptidase 4; SGLT2: Sodium/glucose cotransporter 2; GLP-1: Glucagon-like peptide 1; NLR_{max}: Maximum neutrophil:lymphocyte ratio; CRP: C-reactive protein; CRP_{max}: Maximum C-reactive protein.

via the production of mitochondrial reactive oxygen species and activation of hypoxia-inducible factor 1 α[12]. Further, individuals suffering from diabetes are thought to have chronic low-grade inflammation, which might facilitate the cytokine storm that can lead to clinical deterioration of COVID-19 patients [13]. In addition, patients with diabetes have impaired NK cell activity and altered T cell subpopulations that may increase the susceptibility to severe COVID-19 pneumonia[5,6,9,13,14]. Moreover, other risk factors for severe COVID-19 pneumonia are associated with diabetes, *e.g.*, obesity, CVD, and CKD[5,8, 10]. Therefore, in the current study, we focused on patients with type 2 diabetes who were hospitalized at the Galilee Medical Center due to COVID-19 infection or other acute diseases for comparison.

Interestingly, baseline albuminuria was more common in patients without COVID-19 infection (54.9% compared to 30.8%, $P = 0.005$). The observed low rate of albuminuria in the COVID-19 group led us to explore the associations of several baseline clinical variables, including baseline albuminuria and

Table 3 Clinical and demographic characteristics of patients with type 2 diabetes, with mild or moderate versus severe or critical coronavirus disease 2019 pneumonia

	Mild-moderate COVID-19 pneumonia, *n* = 34	Severe-critical COVID-19 pneumonia, *n* = 31	*P* value
Age (yr), mean (SD)	58.6 (10.0)	61.2 (8.1)	0.26[1]
Median (IQR)	59.5 (50.8-65.3)	63 (57.0-67.0)	
Sex, female, *n* (%)	20 (58.8)	12 (38.7)	0.14[2]
Population group, *n* (%)			1.00[2]
Jews	10 (29.4)	10 (32.3)	
Arabs	24 (70.6)	21 (67.7)	
BMI (kg/m^2), mean (SD)	31.4 (3.8)	32.8 (5.2)	0.22[1]
Median (IQR)	31.2 (28.9-33.6)	31.7 (29.3-36.1)	
Diabetes duration (yr), median (IQR)	9.5 (4.8-12.5)	11 (7-17)	0.13[3]
eGFR (mL/min/1.73 m^2 body surface area) at baseline, median	90.4 (74.9-105.5)	93.3 (76.0-100.3)	0.78[3]
HbA1C (%), median (IQR)	7.4 (6.6-9.1)	7.4 (6.6-9.4)	0.93[3]
Metformin, *n* (%)	30 (88.2)	26 (83.9)	0.73[4]
DPP-4, *n* (%)	10 (29.4)	8 (25.8)	0.79[2]
Sulfonylurea, *n* (%)	5 (14.7)	2 (6.5)	0.43[4]
SGLT2 inhibitors, *n* (%)	11 (32.4)	7 (22.6)	0.42[2]
GLP-1 agonists, *n* (%)	5 (14.7)	7 (22.6)	0.53[2]
Basal insulin, *n* (%)	8 (23.5)	10 (32.3)	0.58[2]
Prandial insulin, *n* (%)	3 (8.8)	3 (9.7)	1.00[4]
Current smoking, *n* (%)	4 (11.8)	0 (0)	0.12[4]
No albuminuria, *n* (%)	21 (61.8)	24 (77.4)	0.19[2]
			2-sided
			0.14[2]
			1-sided
Albuminuria < 30 mg/g, *n* (%)	13 (38.2)	7 (22.6)	0.145[3]
			2-sided
Albuminuria 30-300 mg/g, *n* (%)	8 (23.5)	6 (19.4)	0.073[3]
Albuminuria > 300 mg/g, *n* (%)	5 (14.7)	1 (3.2)	1-sided
Retinopathy, *n* (%)	4 (16.7)	3 (13.6)	1.00[4]
NLR_{max} median (IQR)	2.7 (1.7-8.5)	9.2 (5.7-15.4)	< 0.001[3]
CRP (mg/L) at admission, median (IQR)	15.4 (5.1-80.9)	122.1 (70.8-177.2)	< 0.001[3]
CRP_{max} (mg/L) at hospitalization, median (IQR)	15.4 (5.1-86.2)	142.3 (87.1-205.1)	< 0.001[3]
eGFR (mL/min/1.73 m^2 body surface area) at hospitalization, median (IQR)	91.2 (76.0-105.0)	93.3 (76.5-100.3)	0.90[3]
Albumin (g/dL), mean (SD)	3.8 (0.4)	3.3 (0.4)	< 0.001[1]
Median (IQR)	3.9 (3.6-4.1)	3.3 (3.1-3.5)	

[1]Independent sample *t*-test.
[2]Chi-square test.
[3]Mann-Whitney test.
[4]Fisher's exact test.
COVID-19: Coronavirus disease 2019; IQR: Interquartile range; BMI: Body mass index; HbA1c: Glycosylated hemoglobin; eGFR: Estimated glomerular

filtration rate; DPP-4: Dipeptidyl-peptidase 4; SGLT2: Sodium/glucose cotransporter 2; GLP-1: Glucagon-like peptide 1; NLR_{max}: Maximum neutrophil:lymphocyte ratio; CRP: C-reactive protein; CRP_{max}: Maximum C-reactive protein.

Table 4 Multivariable logistic regression analysis performed to determine the risk factors for severe coronavirus disease 2019 infection (*n* = 65)

		OR	95%CI	*P* value
Model 1	Sex (female *vs* male)	0.30	0.10-0.93	0.038
Baseline characteristics	Age (yr)	1.03	0.98-1.09	0.28
R-square: 14.4%	BMI (kg/m^2)	1.12	0.99-1.28	0.073
Model 2	Sex (female *vs* male)	0.57	0.15-2.16	0.41
Baseline characteristics	Age (yr)	1.02	0.94-1.11	0.62
R-square: 34.4%	BMI (kg/m^2)	1.14	0.96-1.35	0.13
	HbA1c (%)	0.85	0.59-1.23	0.39
	Baseline eGFR (mL/min/ 1.73 m^2 of body surface area)	1.02	0.98-1.07	0.28
	NLR_{max}	1.18	1.05-1.33	0.007
Model 3	Sex (female *vs* male)	0.42	0.09-1.94	0.27
Baseline characteristics	Age (yr)	1.08	0.97-1.20	0.18
R-square = 46.9%	BMI (kg/m^2)	1.24	1.01-1.53	0.040
	HbA1c (%)	1.04	0.67-1.61	0.89
	Baseline eGFR (mL/min/1.73 m^2 of body surface area)	1.04	0.99-1.09	0.12
	NLR_{max}	1.20	1.06-1.37	0.005
	Albuminuria	0.09	0.01-0.62	0.015

OR: Odds ratio; CI: Confidence intervals; BMI: Body mass index; HbA1c: Glycosylated hemoglobin; eGFR: Estimated glomerular filtration rate; NLR_{max}: Maximum neutrophil:lymphocyte ratio; CRP: C-reactive protein; IQR: Interquartile range.

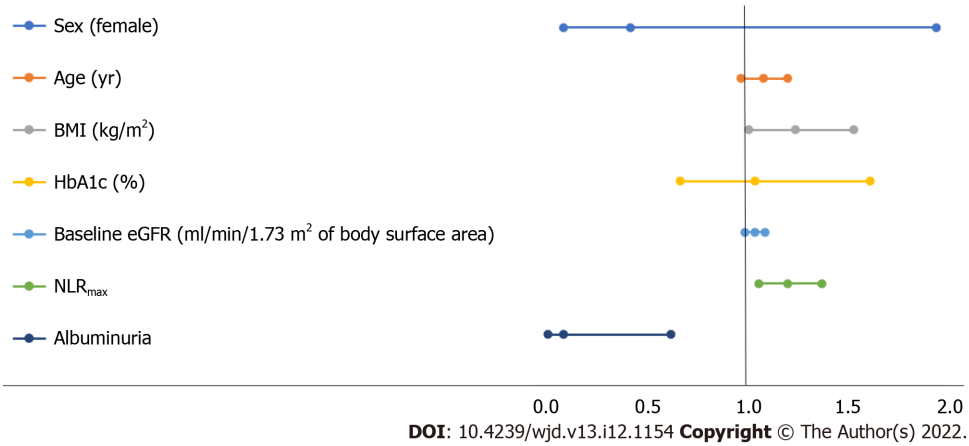

Figure 2 Multivariable logistic regression Model 3 as shown in Table 4. The dependent variable is the severity of coronavirus disease 2019 pneumonia, while the following parameters are independent variables: age, sex, body mass index, last estimated glomerular filtration rate measured before hospitalization, glycosylated hemoglobin, maximum neutrophil:lymphocyte ratio, and albuminuria before hospitalization. BMI: Body mass index; eGFR: Estimated glomerular filtration rate; HbA1c: Glycosylated hemoglobin; NLR_{max}: Maximum neutrophil:lymphocyte ratio.

antiglycemic therapies, as predictors of severe COVID-19 pneumonia. Similarly to previous publications, we identified factors that were significantly associated with increased severity of COVID-19 pneumonia[3-5], including higher BMI (OR 1.24, 95%CI: 1.01-1.53, *P* = 0.04) and NLR_{max} (OR 1.2, 95%CI:

Table 5 Multivariable logistic regression analysis performed to determine the risk factors for bacterial infection (*n* = 144)

		OR	95%CI	*P* value
Model 1	Sex (female *vs* male)	1.88	0.70-5.05	0.21
Baseline characteristics	Age (yr)	0.98	0.93-1.02	0.34
R-square: 2.8%	BMI (kg/m^2)	0.95	0.88-1.04	0.27
Model 2	Sex (female *vs* male)	7.23	1.57-33.23	0.011
Baseline characteristics	Age (yr)	1.00	0.92-1.09	0.97
R-square: 54.0%	BMI (kg/m^2)	0.94	0.84-1.05	0.26
	HbA1c (%)	1.19	0.88-1.60	0.25
	Baseline eGFR (mL/min/1.73 m^2 of body surface area)	1.05	1.01-1.08	0.013
	NLR_{max}	1.36	1.21-1.54	< 0.001
Model 3	Sex (female *vs* male)	7.26	1.58-33.44	0.011
Baseline characteristics	BMI (kg/m^2)	0.94	0.84-1.05	0.26
R-square: 54.0%	Age (yr)	1.00	0.92-1.09	0.98
	HbA1c (%)	1.19	0.87-1.61	0.28
	Baseline eGFR (mL/min/1.73 m^2 of body surface area)	1.05	1.01-1.08	0.012
	NLR_{max}	1.37	1.21-1.55	< 0.001
	Albuminuria	1.12	0.32-3.96	0.86

OR: Odds ratio; CI: Confidence intervals; BMI: Body mass index; HbA1c: Glycosylated hemoglobin; eGFR: Estimated glomerular filtration rate; NLR_{max}: Maximum neutrophil:lymphocyte ratio.

1.06-1.37, P = 0.005), the latter reflecting the immune modulation caused by COVID-19 viremia. Surprisingly, the presence of moderate-range albuminuria before hospitalization was associated with reduced risk for severe COVID-19 pneumonia (OR 0.09, 95%CI: 0.01-0.62, P = 0.015).

Several oral antiglycemic therapies may yield protective anti-COVID-19 properties due to their pleotropic effects. Given that DPP-4 is thought to be one of the COVID-19 receptors and DPP-4 inhibitors have anti-inflammatory activity[15], these medications were indeed associated with a better clinical outcome in COVID-19 patients[8,16]. GLP-1 receptor agonists have several immune modulation activities, including inhibition of nuclear factor-κB[5,17]. SGLT2 inhibition decreases the mRNA expression levels of some cytokines and chemokines, such as tumor necrosis factor, interleukin-6, and monocyte chemoattractant protein 1[14]. Metformin treatment reduces the circulating levels of inflammatory biomarkers in diabetics, and was associated with significantly lower in-hospital mortality in a retrospective study of COVID-19 patients[5,18,19]. Despite the above properties, in the current study we did not detect a protective effect of any class of antiglycemic medication. It is possible that the current cohort was underpowered to detect such differences.

The protective association of baseline moderate-range albuminuria with severe COVID-19 pneumonia was unexpected and counterintuitive, given the well-established role of CKD in enhancing severity of the disease. This association was not observed in patients suffering from bacterial infection, suggesting a specific protective effect against COVID-19, as we cannot draw any conclusions about other viral pathogens in the current cohort. To our knowledge, baseline moderate-range albuminuria with eGFR > 60 mL/min has not been described as conferring such an advantage as demonstrated here, and we cannot draw any conclusions about other viral pathogens in the current cohort.

How can we reconcile this protective association between baseline albuminuria and reduced risk for severe COVID-19 pneumonia?

Post-mortem findings in the lungs of people with fatal COVID-19 demonstrated diffuse alveolar damage and inflammatory cell infiltration[20]. The inflammatory response in patients with severe COVID-19 pneumonia is impaired. Specifically, it has been demonstrated that interferon (IFN) type I response is disrupted with low IFNα activity in the blood, indicating high blood viral load and an impaired inflammatory response[21]. Despite IFN's protective role in COVID-19 infection, IFN can lead to proteinuria. The role of activation of type I IFN signaling in mediating proteinuria is well-known. Podocytes respond to toll-like receptor ligand-like polyinosinic:polycytidylic acid [poly (I:C)] that simulates viral infection, by releasing pro-inflammatory cytokines and activation of type I IFN signaling. IFN signaling enhances podocyte B7-1 expression and actin remodeling *in vitro* and leads to

transient proteinuria *in vivo*. Interestingly, mice treated with a type I IFN receptor-blocking antibody were protected from lipopolysaccharide-induced proteinuria[22]. Therefore, we hypothesize that the presence of moderate-range albuminuria may represent intact type 1 IFN signaling, and in the case of COVID-19 infection, such intact IFN signaling can confer protection from severe COVID-19 pneumonia. We emphasize that moderate-range albuminuria has a protective association in the context of patients with mild CKD (eGFR > 60 mL/min), who constitute the majority of participants in our cohort. Notwithstanding, it was recently demonstrated that patients with eGFR < 60 mL/min or severe albuminuria are at risk for severe COVID-19 infection[23], probably due to advanced CKD contributing to impaired immune function.

A similarly counterintuitive association was recently demonstrated in a cohort of inflammatory bowel disease (IBD) patients. Severe sequelae of COVID-19 were lower in IBD patients compared to matched non-IBD controls, suggesting that baseline immune activity may modulate the progression of COVID-19 pneumonia[24]. In addition, the unexpected finding with regard to moderate-range albuminuria and protection from severe COVID-19 pneumonia might be the result of confounding by as yet unidentified factors or collider bias.

As with all observational studies, our study has limitations. We did not have information regarding other co-morbidities such as liver disease, respiratory disease, alcohol abuse, cognitive impairment, *etc.*, which can potentially serve as confounding factors. Residual confounding might also have resulted from the use of only several measurements to identify baseline characteristics. We did not have data about urine albumin:creatinine during hospitalization; therefore, the contribution of this variable was not included in the final model. In addition, we did not have baseline clinical data for patients who did not survive COVID-19 infection, as described in the methods relating to patient enrollment. Of note, the sample size was small, but was adequate to demonstrate statistical significance of the protective association between moderate-range albuminuria and severe COVID-19 pneumonia.

CONCLUSION

The presence of moderate-range albuminuria in patients with diabetes suffering from COVID-19 pneumonia may represent an intact IFN type 1 response that may translate to protection against severe disease. Further studies are needed to explore whether this association is also relevant to other viral infections and characterize the pathogenesis relating to immunomodulation, which may indicate a biomarker for patients at reduced risk for COVID-19-related deterioration that may translate to therapeutic interventions.

ARTICLE HIGHLIGHTS

Research background

Several risk factors for severe coronavirus disease 2019 (COVID-19) disease have been described, including: Advanced age, male sex, smoking history, and underlying chronic diseases such as cardiovascular disease, diabetes mellitus, obesity, underweight, and chronic kidney disease (CKD). This case-control study was conducted to identify risk factors for severe COVID-19 pneumonia in patients with type 2 diabetic mellitus hospitalized at the Galilee Medical Center (Nahariya, Israel).

Research motivation

We aimed to characterize differences between hospitalized diabetic patients with *vs* patients without COVID-19, and parameters associated with COVID-19 severity for prediction.

Research objectives

Similar to previous reports, multivariable logistic regression showed higher body mass index (BMI) and neutrophil:lymphocyte ratio (NLR) were significantly associated with severe COVID-19. Surprisingly, pre-hospitalization albuminuria, mostly moderate-range (albumin:creatinine 30-300 mg/g), was associated with reduced risk for severe COVID-19 pneumonia. The counterintuitive protective association in patients with stage II CKD was not described before. Given the causative association between type I interferon (IFN) signaling and proteinuria, we hypothesize that the presence of moderate-range albuminuria may represent an intact type I IFN signaling, which confers protection from severe COVID-19 pneumonia and its complications.

Research methods

This case-control study included 209 patients with type 2 diabetic mellitus hospitalized at the Galilee Medical Center (Nahariya, Israel) and recruited between September 2020 and May 2021, 65 patients with COVID-19 infection in dedicated wards and 144 COVID-19-negative patients in internal medicine

wards hospitalized due to other reasons. Clinical parameters – including age, type of antiglycemic medications, presence of retinopathy, smoking history, BMI, glycosylated hemoglobin, maximum NLR (NLR_{max}), C-reactive protein (CRP_{max}), estimated glomerular filtration rate (eGFR), and albumin (blood and urine) - were compared between the two primary patient groups, and then between COVID-19-negative patients hospitalized due to infectious *vs* non-infectious disease. Finally, we explored which parameters were associated with severe COVID-19 pneumonia.

Research results

COVID-19-negative patients were older and had longer duration of diabetes, lower eGFR, higher albumin, lower CRP, greater smoking history, and more baseline albuminuria at admission. 70% of COVID-19 patients with albuminuria had moderate-range albuminuria. Most of the patients with albuminuria had CKD II. Oral antiglycemic therapies were not significantly different between the two groups. As previously reported, multivariable logistic regression showed higher BMI and higher NLR were significantly associated with severe COVID-19. Surprisingly, pre-hospitalization albuminuria, mostly moderate-range, was associated with reduced risk for severe COVID-19 pneumonia. This protective association was specific to COVID-19 infection and was not observed in bacterial infections.

Research conclusions

Moderate-range albuminuria in COVID-19-positive diabetic patients with CKD II is associated with less severe COVID-19. We hypothesize that this counterintuitive association may represent intact IFN signaling that on the one hand can lead to harmful proteinuria *via* podocyte injury, and on the other hand can serve as a protective cytokine with the potential to mitigate COVID-19 infection and complications. Given the importance of intact type I IFN response in controlling COVID-19, we suggest that moderate-range albuminuria in diabetic patients with mild CKD may serve as a biomarker for intact IFN signaling and therefore is associated with reduced risk for severe COVID-19 pneumonia.

Research perspectives

Further studies should explore the potential role of albuminuria in the presence of mild CKD as a biomarker for reduced risk of COVID-19-related deterioration that may translate to therapeutic interventions.

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FOOTNOTES

Author contributions: Bashkin A was the guarantor and designed the study, and was responsible for conceptualization, project administration, supervision, methodology, writing, review and editing; Shehadeh M and Shbita L were responsible for data curation and participated in formal analysis; Namoura K, Haiek R, Boulos Y, and Kuyantseva E participated in data curation; Yakir O was responsible for formal analysis; Kruzel-Davila E was responsible for methodology, formal analysis, writing, review and editing.

Institutional review board statement: The study was reviewed and approved by the Helsinki Committee of the Galilee Medical Center.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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SYSTEMATIC REVIEWS

Comparison of gliclazide *vs* linagliptin on hypoglycemia and cardiovascular events in type 2 diabetes mellitus: A systematic review

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Abstract

BACKGROUND

Cardiovascular outcome trials have demonstrated cardiovascular safety of glimepiride (a sulfonylureas) against dipeptidyl peptidase-4 inhibitor linagliptin. Gliclazide (another newer sulfonylureas) has shown similar glycemic efficacy and 50% decreased risk of hypoglycemia compared to glimepiride.

AIM

Considering the absence of cardiovascular outcome trials for gliclazide, we decided to conduct a systematic review of the literature to assess the cardiovascular (CV) safety by assessing the risk for major adverse CV events and hypoglycemia risk of gliclazide *vs* linagliptin in patients with type 2 diabetes (T2D).

METHODS

This systematic review followed the current Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines to analyze all the clinical studies published from 2008 that compared the two drugs in patients with T2D with no risk of CV disease (CVD). We included only evidence designated high quality by the Oxford Center for Evidence-based Medicine-Levels of Evidence.

RESULTS

Eight clinical studies were included in the narrative descriptive analysis

(gliclazide: 5 and linagliptin: 3). The CV safety of gliclazide in the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation trial and of linagliptin in the Cardiovascular and Renal Microvascular Outcome Study With Linagliptin (CARMELINA) and CARdiovascular Outcome study of LINAgliptin *vs* glimepiride in patients with T2D (CAROLINA) trials were excluded from the comparative analysis as these trials demonstrated CV and hypoglycemia benefits in patients at high risk of CVD. However, since these are landmark trials, they were discussed in brief to show the CV benefits and low hypoglycemia risk of gliclazide and linagliptin. We did not find any study comparing gliclazide with linagliptin. Hence, direct comparison of their major adverse CV events and hypoglycemia risk could not be carried out. However, the literature meeting the inclusion criteria showed that both drugs were effective in achieving the desired glycemic control and had low major adverse CV events and hypoglycemia risk in adult patients with no history of CVD.

CONCLUSION

Gliclazide can be considered an effective and safe glucose-lowering drug in T2D patients with no established CVD but at high risk of CVD due to their T2D status. Future randomized controlled trials comparing gliclazide with linagliptin or dipeptidyl peptidase-4 inhibitors can confirm these findings.

Key Words: Linagliptin; Gliclazide; Hypoglycemia; Major cardiovascular adverse events; Type 2 diabetes

Core Tip: This systematic review showed the lack of high-quality evidence and head-to head trials comparing the cardiovascular safety and hypoglycemia risk of gliclazide (a sulfonylurea) *vs* linagliptin (dipeptidyl peptidase-4 inhibitor) in adults with type 2 diabetes and no cardiovascular disease. While dipeptidyl peptidase-4 inhibitors have been proven to be cardiovascular neutral, sulfonylureas like gliclazide are commonly prescribed and recommended glucose-lowering drugs in low resource settings. Hence, it is important to establish the cardiovascular safety and hypoglycemia risk of gliclazide *vs* linagliptin to highlight that gliclazide may be a cost-effective yet safe treatment option for patients with type 2 diabetes.

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INTRODUCTION

Type 2 diabetes (T2D), characterized by chronic hyperglycemia and impaired insulin secretion, is often associated with disease-related microvascular and macrovascular complications and treatment-related complications like hypoglycemia[1,2]. Consequently, patients with T2D are at an increased risk for cardiovascular (CV) complications and hypoglycemia. Hence, glucose-lowering drugs (GLDs) should not have CV complications and higher hypoglycemic episodes (HE) as adverse effects (AEs) and should ideally provide CV benefits or neutrality[1,2].

Sulfonylureas (SUs) are the most prescribed T2D pharmacotherapy, especially in resource limited settings[3]. Apart from their cost benefit, Sus have an exceptional glycemic efficacy with average glycosylated hemoglobin (HbA1c) reduction by 1%-2%, good safety profile and gastrointestinal tolerability[3]. However, hypoglycemia, weight gain and decreasing efficacy over time are the main concerns with SUs due to their insulinotropic mechanism of action[3-5]. On the other hand, newer oral GLDs like dipeptidyl peptidase-4 (DPP4) inhibitors and sodium-glucose cotransporter-2 (SGLT2) inhibitors provide comparably less glycemic control than SUs (average HbA1c reduction 0.5%-0.8%), are costlier than SUs and often need to be combined with SUs to achieve the required glycemic control[3].

However, since, the time of their inception into T2D treatment regime, SUs have been subjected to criticism for CV safety[3,6]. The CV safety of SUs has been derived from small, inadequately powered randomized controlled trials (RCTs) and observational studies[3]. However, formal cardiovascular outcome trials (CVOTs) are not available for SUs[3,6].

Then, in 2008, the United States Food and Drug Administration mandated the assessment of CV safety of newer GLDs[7]. Hence, large multinational, CVOTs of newer oral GLDs like DPP4 inhibitors[8-12] and SGLT2 inhibitors[13-15] were conducted and showed their CV benefits. DPP4 inhibitors and SGLT2 inhibitors proved to be costly options in resource limited settings because of the chronic disease nature of T2D and because most patients pay from their pocket for the treatment[16,17].

Despite their unquestionable glucose lowering efficacy, current diabetes guidelines no longer favors the use of SUs because of CV safety concerns except when cost is an issue[3,6]. SUs have been recommended as the add-on of choice after metformin for adequate glycemic control in resource limited settings by the World Health Organization (WHO) Guidelines, the Research Society for the Study of Diabetes in India/Endocrine Society of India (RSSDI-ESI) (2020) guidelines from India[18,19], the International Task Force (ITF) Consensus[20] and the International Diabetes Federation (IDF)[21]. The ITF recommends glimepiride and gliclazide modified release (MR) as the SU of choice to be added to metformin, while the IDF gave equal importance to SUs (except glibenclamide/glyburide), a DPP4 inhibitor or an SGLT2 inhibitor[20,21].

The American Diabetes Association (ADA) (2021) guidelines recommend various add-on pharmacotherapies for T2D patients poorly controlled on metformin, including DPP4 inhibitors, SGLT2 inhibitors and SUs[22]. The American Diabetes Association guidelines recommend T2D patients with CV and renal morbidities should ideally be prescribed SGLT2 inhibitors or glucagon-like peptide-1 (GLP-1) agonists as the next oral GLDs after metformin[22]. However, the choice of add-on therapy in patients without CV risk is not clear.

Of the various DPP4 inhibitors used in T2D, landmark linagliptin trials have demonstrated CV safety and safety against HE in T2D patients with a high risk of CV disease (CVD)[8,9]. On the other hand, a landmark non-CVOT trial in patients with high CV risk showed that high intensity gliclazide treatment conferred low CV risk[23].

Many systematic reviews (SRs) and/or meta-analyses (MAs) have assessed the efficacy and safety [hypoglycemia and major adverse cardiovascular events (MACE; CV death, nonfatal myocardial infarction/ischemia/acute coronary syndrome or nonfatal stroke)] of SUs *vs* DPP4 inhibitors with mixed results[24-28]. These SRs and meta-analyses identified a need for RCTs comparing individual SUs with a DPP4 inhibitor. Hence, this SR was carried out to assess the CV safety and hypoglycemia risk of gliclazide *vs* linagliptin in T2D patients, both in monotherapy and as add-on to metformin setting.

MATERIALS AND METHODS

Methodology

The MEDLINE database was searched on September 9, 2021 for records on gliclazide or linagliptin with no filter added. This retrieved 2578 records. An advanced search filter was then applied to filter by English language only, clinical trials, RCT, human studies and adult age (19 + years). These filters retrieved 2054 records. The records were further filtered by applying adverse events of interest: hypoglycemia, low blood sugar, myocardial infarction/myocardial ischemia (MI), transient ischemic attack, CV death and stroke. This retrieved 615 records; 223 duplicates were removed and the remaining 392 records were screened. It was seen that linagliptin records were available from 2008 onwards only. Hence, to standardize the time period for the entire literature search, gliclazide records published before 2008 were removed. The remaining 248 records were assessed for eligibility. After excluding records that did not meet the eligibility criteria as mentioned in Table 1, eight records were included (5 for gliclazide and 3 for linagliptin). The details of the literature search and study selection are outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart (Figure 1). Google Scholar was searched for any additional manuscripts that were missed on MEDLINE. This retrieved no additional records as per study selection criteria.

Two independent reviewers used the current PRISMA guidelines for SRs[29,30] to independently carry out the literature search on the same day. Any conflict in the number of records at identification, screening, eligibility and inclusion were mutually discussed and resolved by consensus. We do note that the protocol for this systematic review has not been published.

Quality of evidence and risk of bias

As shown by the PRISMA flow chart, there were many articles regarding both gliclazide and linagliptin. Hence, we included only high-quality evidence. RCTs were designated the highest quality by the Oxford Center for Evidence-based Medicine-Levels of Evidence[31] followed by a randomized design of any type. Hence, we included only randomized studies. Placebo controlled studies were not included as there were no gliclazide *vs* placebo studies. The main reason for this could be that trials in the initial trajectory of drug development were missed by standardizing the study period from 2008 onwards. Additionally, studies comparing gliclazide or linagliptin with metformin were also not included because both drugs have a known and comparable efficacy and safety profile *vs* metformin.

Table 1 Inclusion and exclusion criteria of the records included in the systematic review

Inclusion criteria	Exclusion criteria
Age 19 yr and < 70 yr; Male and Female; type 2 diabetes	Age below 19 yr or ≥ 70 yr; type 1 diabetes; no diabetes
Human studies: Any race, ethnicity	Clinical trials evaluating gliclazide or linagliptin in patients with specific comorbidites including CVD[1]
Randomized clinical trials on safety of: -Gliclazide monotherapy versus linagliptin monotherapy -Gliclazide + metformin versus linagliptin + metformin	Review articles, systematic reviews and meta-analysis, network meta-analysis, pooled analysis of trials, case studies, non-randomized trials
Randomized clinical trials on safety of: -Gliclazide versus DPP4 inhibitors -Linagliptin versus sulfonylureas	Pharmacokinetic, pharmacodynamic and bioequivalence study; retrospective chart review; observational real-world study; case study; trials studying mechanism of action of gliclazide or linagliptin; literature reporting only study design; trial summaries and implications; animal studies; preclinical studies
Randomized clinical trials on gliclazide or linagliptin monotherapy evaluating the following outcomes:	Clinical trials evaluating gliclazide or linagliptin versus PBO
-Hypoglycemia or low blood sugar	Clinical trials evaluating gliclazide or linagliptin in combination with other GLDs except metformin
-Occurrence of 3 point major adverse cardiovascular events (3P-MACE): Cardiovascular death, nonfatal myocardial infarction/ischemia/acute coronary syndrome, or nonfatal stroke (transient ischemic attack included)	Clinical trials evaluating gliclazide or linagliptin versus other GLDs except: (1) DPP4 inhibitors for gliclazide; and (2)sulfonylureas for linagliptin
	Clinical trials evaluating other glycemic, cardiac, cardiovascular outcomes than those of interest; other outcomes (*e.g.*, microvascular complications)

[1]History of myocardial infarction, stroke, unstable angina, transient ischemic attack, percutaneous coronary intervention for coronary occlusion or coronary artery bypass graft.

Note: Efficacy was synthesized from the gliclazide and linagliptin studies that met the inclusion criteria. CVD: Cardiovascular disease; DPP4: Dipeptidyl peptidase-4; GLD: Glucose-lowering drugs; PBO: Placebo.

Further, risk of bias assessment was independently carried out by two researchers who assessed the scientific quality of the records using the Cochrane Collaboration's tool for risk of bias assessment[32]. The Cochrane Risk of Bias tool assesses seven domains of bias and stratifies the risk of bias as low, high and unclear risk. Discrepancies between reviewers at any stage were resolved by discussion and consensus.

All the studies clearly defined and reported the outcomes of interest (hypoglycemia and MACE) and clearly mentioned all the CVDs that were assessed as exclusion criteria. Only one gliclazide trial[33] did not have any CVD as an exclusion criteria. The trials clearly explained the randomization schedule and were largely double-blind studies. The number of participants for which the outcomes of interest were reported was clearly stated.

However, most studies were not designed to report the outcome of interest (hypoglycemia and MACE) as their main primary and/or secondary endpoint. These outcomes of interest were primarily reported as AEs or safety endpoints.

Statistical analysis

The systematic literature search (Figure 1) did not retrieve any head-to-head trials comparing gliclazide ± metformin with linagliptin ± metformin. Hence, direct comparison of their outcomes was not possible. The gliclazide and linagliptin trials that met the inclusion criteria could not be compared to reach a statistical analysis due to various reasons. The studies captured for the two drugs were heterogeneous with respect to study design and duration, the outcomes of interest being evaluated as primary or secondary or safety (as AE) endpoints or as incident findings, definition of outcomes [*e.g.*, definition of hypoglycemia-cut off blood glucose (BG) level] and the statistical method used for analysis. The study population of the various studies differed in age, ethnicity and patient profile (*e.g.*, treatment naïve or after failure of SU). Hence, a meta-analysis or a network meta-analysis could not be carried out. Therefore, key outcomes were described in a narrative manner for each drug separately, with due consideration given to the PRISMA checklist[29].

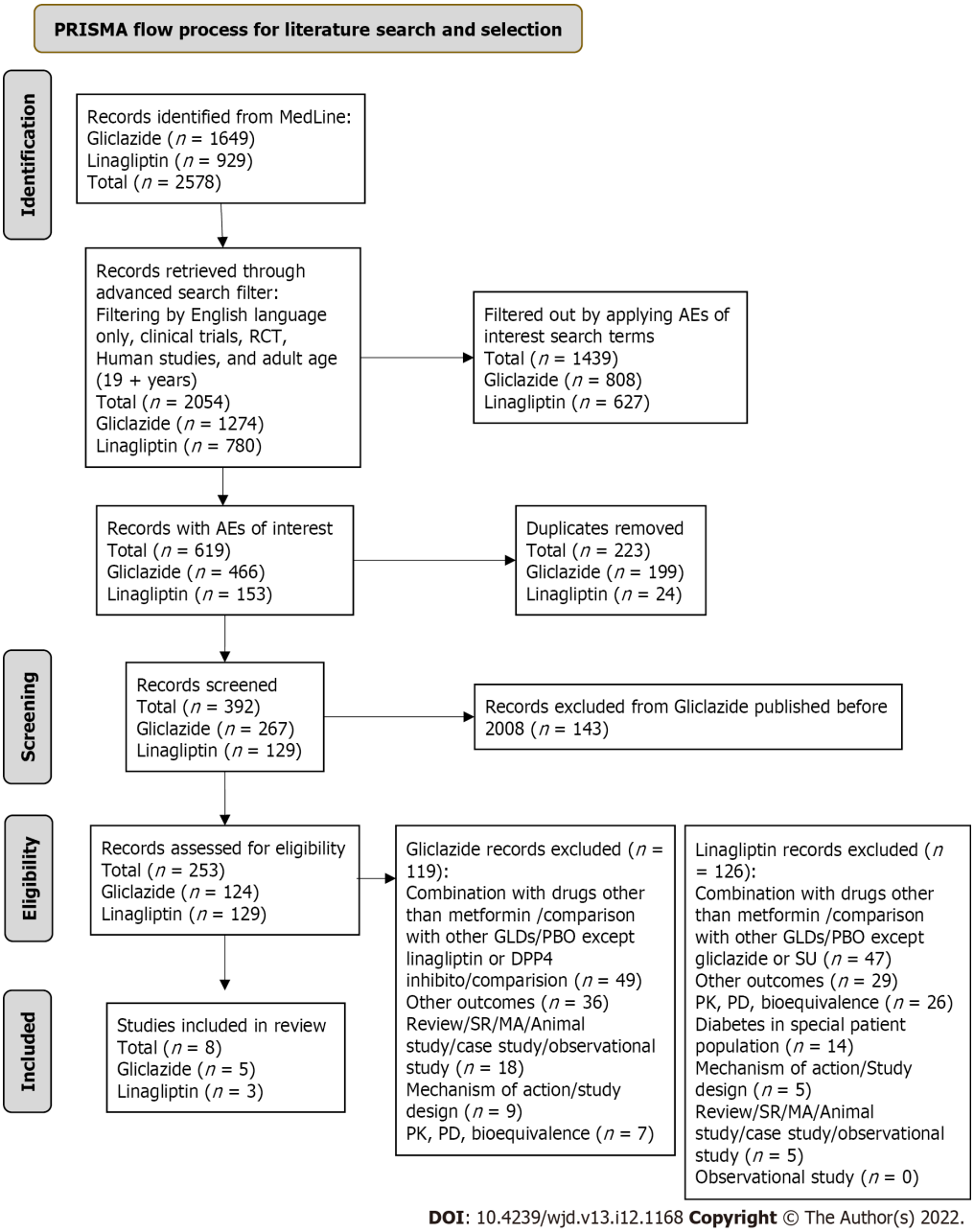

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Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow chart of literature search and selection. AE: Adverse event; DPP4: Dipeptidyl peptidase 4; GLD: Glucose-lowering drug; MA: Meta-analysis; PBO: Placebo; PD: Pharmacodynamic; PK: Pharmacokinetic; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RCT: Randomized controlled trials; SR: Systematic review; SU: Sulfonylurea.

RESULTS

Gliclazide studies

This section aimed to include RCTs that compared gliclazide *vs* linagliptin or a DPP4 inhibitor in a monotherapy setting or compared gliclazide as an add-on to metformin *vs* linagliptin/DPP4 inhibitor as add-on to metformin.

Gliclazide *vs* linagliptin or DPP4 inhibitors: There were no records comparing gliclazide with linagliptin. One study compared gliclazide with vildagliptin, a DPP4 inhibitor[34] (Table 2). Foley *et al* [34] compared the efficacy and safety of 2 years of monotherapy with vildagliptin *vs* gliclazide in 1092 drug-naïve patients with T2D having HbA1c of 7.5%-11.0%. In this vildagliptin non-inferiority trial, the vildagliptin group had a lower incidence of grade 1 hypoglycemia than the gliclazide group (0.7% *vs* 1.7%).

Two patients in the gliclazide group and none in the vildagliptin group had ≥ 2 HEs[34]. Though the baseline HbA1c values were slightly higher in the group treated with gliclazide *vs* the vildagliptin group (HbA1c of 8.7% ± 0.1% *vs* 8.5% ± 0.1%), the mean HbA1c reduction from baseline to week 104 was

Table 2 Gliclazide *vs* dipeptidyl peptidase-4 inhibitor /linagliptin *vs* sulfonylurea

Ref.	Primary study objective	Study design	Study population	CVD excluded	Number of participants	Study duration	Endpoint (hypoglycemia)	Hypoglycemia definition	Hypoglycemia results	Endpoint (MACE)	MACE definition	MACE results
Gliclazide *vs* DPP4 inhibitor (vildagliptin)												
Foley *et al* [34], 2009	Compare the efficacy and safety of vildagliptin *vs* gliclazide	Randomized, multicenter, double-blind, active-controlled study	Drug-naïve patients with T2D, HbA1c of 7.5%-11.0%	CHF NYHA class III or IV, ECG abnormalities	1092	104 wk	AEs were safety endpoints	Grade 1 hypoglycemic events per week: symptoms suggestive of low blood glucose confirmed by SMBG measurement of < 3.1 mmol/L plasma glucose equivalent not requiring the assistance of another party; Grade 2 hypoglycemic event (requiring the assistance of another party) or if there were 3 or more asymptomatic glucose values < 3.1 mmol/L per week	Grade 1 hypoglycemia: 4 patients (0.7%) in the vildagliptin group and 14 (1.7%) in the gliclazide group. ≥ 2 HEs: 2 patients in the gliclazide group and none in vildagliptin group No grade 2 HEs in either group	-	-	-
Gliclazide + metformin *vs* DPP4 inhibitor (vildagliptin) + metformin												
Vianna *et al* [35], 2018 (Part of BoneGlic Trial)	Compare the effects on glycemic variability and bone metabolism	Single center, randomized, controlled, open-label (blinded to the observer)	Postmenopausal Brazilian women with T2D and treated with a stable metformin dose for ≤ 3 mo	CV complications	56 (42 randomized)	2-wk pre-randomization period followed by 24 wk	As AE	Major hypoglycemia: glucagon, carbohydrates administration by another person or other resuscitative measures; minor hypoglycemia: BG ≤ 3.9 mmol/L with or without typical symptoms or hypoglycemia symptoms without BG test	No differences from baseline in time to hypoglycemia (% of time ≤ 3.9 mmol/L) No major hypoglycemia Minor hypoglycemia events: 7 in the gliclazide; 2 in the vildagliptin group (P = 0.062)	As SAE		Vildagliptin: 1 hemorrhagic stroke gliclazide MR group: 1 death due to AMI, the investigator did not consider the SAEs to be related to the study medications
Hassanein *et al*[36], 2014 (STEADFAST study)	HE during Ramadan	Multiregional, randomized double-blind	Patients fasting during Ramadan	CHF (NYHA class III or IV); other significant CV history within 6 mo	557	4-wk Ramadan period	Primary	Hypoglycemia: Low BG symptoms with or without confirmatory, SMBG measurement < 3.9 mmol/L; PGE or asymptomatic SMBG < 3.9 mmol/L PGE; confirmed hypoglycemia: symptomatic/asymptomatic SMBG measurement < 3.9 mmol/L; PGE and severe HE	Confirmed and/or severe HE during Ramadan: vildagliptin *vs* glicalzide was 3.0% *vs* 7.0% (P = 0.039; one-sided test); HEs: vildagliptin *vs* gliclazide was	-	-	-

								requiring assistance from another party irrespective of whether SMBG value was available or not	6.0% and 8.7% (P = 0.173)			
Filozof and Gautier[37], 2010	Demonstrate non-inferiority of vildagliptin compared with gliclazide, as an add-on therapy	Randomized, double-blind, active-controlled	T2D uncontrolled with metformin	Serious cardiac conditions (torsades de pointes, sustained and clinically relevant VT or VF, PCI ≤ 3 mo, MI, CABG, unstable angina, or stroke ≤ 6 mo and CHF requiring pharma-cological treatment, 2nd- or 3rd-degree AV block or prolonged QTc)	1007	52 wk	AE	Symptoms suggestive of hypoglycemia and confirmed by SMBG < 3.1 mmol/L	HE vildagliptin *vs* gliclazide (6 *vs* 11 events)	-	-	-

AE: Adverse event; AMI: Acute myocardial infarction; AV: Atrioventricular; BG: Blood glucose; CABG: Coronary artery bypass surgery; CHF: Congestive heart failure; CV: Cardiovascular; CVD: Cardiovascular disease; DPP4: Dipeptidyl peptidase-4; ECG: Electrocardiogram; HbA1c: Glycated hemoglobin: HE: Hypoglycemia event/episode; MACE: Major adverse cardiovascular event; MI: Myocardial infarction; MR: Modified release; NYHA: New York Heart Association; PCI: Percutaneous coronary intervention; PGE: Plasma glucose equivalent; SAE: Serious adverse event; SMBG: Self-monitored blood glucose; T2D: Type 2 diabetes; VF: Ventricular fibrillation; VT: Ventricular tachycardia.

-0.5% and -0.6% in the vildagliptin *vs* gliclazide group[34]. The study could not show the non-inferiority of the DPP4 inhibitor over gliclazide.

Gliclazide + metformin *vs* linagliptin/DPP4 inhibitors + metformin: There were no records comparing gliclazide + metformin with linagliptin + metformin. Vianna *et al*[35] compared the glycemic variability of gliclazide MR and vildagliptin and their effect on bone metabolism. This study was the single center part of the BoneGlic Trial, which reported hypoglycemia and MACE as AEs in 42 postmenopausal Brazilian women with T2D and treated with a stable metformin dose for ≤ 3 mo. The study found no difference in time to hypoglycemia and the number of HEs in both the groups (P = 0.062). The investigator did not consider MACE events (Table 2) to be related to study drugs.

The study also found that the gliclazide MR group had a significantly longer time within the target BG range [> 3.9 mmol/L and ≤ 10.0 mmol/L (> 70.27 mg/dL and ≤ 180.18 mg/dL)] and a significantly lower percentage of time with BG > 10 mmol/L (180.18 mg/dL) (P = 0.038 and P = 0.029). In comparison, time within the target BG was insignificantly increased and percentage of time with BG > 10 mmol/L (180.18 mg/dL) was insignificantly lower in the vildagliptin group (P = 0.111 and P = 0.133, respectively). However there were no differences between gliclazide and the DPP4 inhibitor for both the parameters[35].

The STEADFAST study conducted on 557 T2D patients fasting during the holy month of Ramadan found that both gliclazide and vildagliptin as add-on therapy was safe[36]. However, confirmed and/or severe HEs during Ramadan were significantly higher (Table 2) in the glicalzide group[36]. The HEs observed with gliclazide were lower than reported from observational studies. The authors of the

STEADFAST study concluded that HEs with gliclazide could be avoided through frequent patient-physician contacts and Ramadan-focused advice[36].

A vildagliptin non-inferiority trial in patients with T2D uncontrolled with metformin demonstrated that as an add-on to metformin, vildagliptin was non-inferior to gliclazide in achieving glycemic control (95% confidence interval: 0.11%-0.20%)[37]. However, more patients in the vildagliptin group discontinued treatment due to an unsatisfactory effect compared with the gliclazide group (n = 22 *vs* 13, respectively). HEs were lower in the vildagliptin group *vs* the gliclazide group (6 events *vs* 11 events)[37].

All three trials[35-37] comparing gliclazide + metformin with DPP4 inhibitor + metformin described in this section were specific to a patient population (post-menopausal women) or in special situation (fasting during Ramadan). Therefore, these trials did not meet the strict inclusion criteria of this narrative synthesis. They were included because there were no other trials retrieved that compared gliclazide with a DPP4 inhibitor as an add-on therapy. The results on these trials may have been influenced by the patient population or the fasting state of the patients.

Linagliptin studies

This section aimed to include randomized trials that compared linagliptin *vs* gliclazide/SU in a monotherapy setting or compared linagliptin as add-on to metformin *vs* gliclazide/SU as add-on to metformin.

Linagliptin *vs* gliclazide or SUs: There were no studies comparing linagliptin with gliclazide or another SU. The landmark "CARdiovascular Outcome study of LINAgliptin *vs* glimepiride in patients with type 2 diabetes" (CAROLINA)[9] trial and studies[38,39] trial did not meet the inclusion criteria of the narrative synthesis as the study primarily focused on the cardiac and renal patient population. Therefore, other studies[38,39] analyzing the outcomes of interest from the CAROLINA trial were also not included in the narrative synthesis. However, this non-inferiority of linagliptin to glimepiride trial merits discussion as it compared linagliptin with an SU, glimepiride. The trial is covered under the excluded trial section.

However, a study by Barnett *et al*[40] in "metformin contraindicated" T2D patients compared linagliptin 5 mg once daily with placebo for 18 wk and then compared linagliptin with glimepiride after weeks 18 for 34 wk. The study defined hypoglycemia according to the 2005 American Diabetes Association guidelines[41]. The linagliptin group experienced less hypoglycemia [≤ 70 mg/dL (≤ 3.9 mmol/L)] (2.2% *vs* 7.8%) and clinical event committee confirmed CV events (0.7% *vs* 1.6%) than the glimepiride group[40]. However, the difference did not reach clinical significance and more patients in the linagliptin group discontinued treatment due to an AE.

Linagliptin + metformin *vs* gliclazide/SU + metformin: The literature search did not retrieve any linagliptin + metformin *vs* gliclazide/SU + metformin studies meeting the inclusion criteria.

Gliclazide/linagliptin ± metformin

The literature search did not retrieve any gliclazide *vs* placebo studies meeting the inclusion criteria. The main reason for this could be that trials in the initial trajectory of drug development were missed by standardizing the study period from 2008 onwards. Also, there were no trials comparing gliclazide ± metformin with linagliptin ± metformin. Hence, this section aimed to include trials evaluating gliclazide alone or gliclazide + metformin without a comparator and linagliptin alone or linagliptin + metformin without a comparator. These trials were then assessed separately to see if the outcomes of interest could be compared.

Gliclazide ± metformin: Only one trial met the inclusion criteria and is detailed in Table 3. The multicenter, randomized, parallel-group "Diamicron MR in NIDDM: Assessing Management and Improving Control" (DINAMIC 1)[33] trial compared the efficacy, tolerability and acceptability of gliclazide MR for T2D management in the self-monitoring of BG (SMBG) *vs* non SMBG group. HEs were reported as a safety outcome and were classified as follows: Grade 1, suspected mild hypoglycemia; grade 2, suspected moderate hypoglycemia; grade 3, suspected severe hypoglycemia with need of third-party assistance; and grade 4, suspected severe hypoglycemia with need of medical assistance. In 610 T2D patients (aged 40-80 years) followed up for 6 mo, 8.7% patients in the SMBG group had a total of 51 HEs and 7.0% of patients in the non-SMBG group had a total of 66 HEs. There were no severe (grade 3 or 4) HEs in any group.

Symptoms suggestive of nocturnal hypoglycemia were experienced by 3 and 7 patients in the SMBG *vs* non-SMBG, respectively. Two patients withdrew from the study because of hypoglycemia, and both were in the non-SMBG group. The study highlighted the importance of SMBG in T2D management.

Linagliptin ± metformin: Only one trial met the inclusion criteria and is detailed in Table 3. This study compared linagliptin + metformin with only linagliptin and hence was included. Ross *et al*[42] conducted a randomized study to evaluate the efficacy and safety of initial treatment with linagliptin/metformin combination in newly diagnosed T2D patients with marked hyperglycemia. Hypoglycemia occurred in 1.9% of patients in the linagliptin/metformin and 3.2% of patients in the

Table 3 Gliclazide ± metformin and linagliptin ± metformin (no comparator)

Ref./treatment	Primary study objective	Study design	Study population	CVD excluded	Number of participants	Study duration	Endpoint (hypoglycemia)	Hypoglycemia definition	Hypoglycemia results	Endpoint (MACE)	MACE definition	MACE results
Barnett *et al*[33], 2008/DINAMIC 1/Gliclazide	Compare the efficacy, tolerability and acceptability of gliclazide in SMBG *vs* non-SMBG group	Multicenter randomized parallel-group	T2D patients managed on diet alone	Not mentioned	610	6 mo	Safety endpoint (AE)	Grade 1: Suspected mild hypoglycemia	SMBG group: 8.7% patients had 51 HE: symptomatic (27), asymptomatic (11), SMBG-confirmed (11) and non-graded (2)	-	-	-
								Grade 2: Suspected moderate hypoglycemia	Non-SMBG group: 7.0% patients had 66 HE: Symptomatic (66) and non-graded (2). Two HE-related withdrawals			
								Grade 3: Suspected severe hypoglycemia with need of third party assistance	No grade 3 or 4 symptoms			
								Grade 4: Suspected severe hypoglycemia with need of medical assistance	Symptoms suggestive of nocturnal hypoglycemia: SMBG group: 3 and non-SMBG group: 7			
Ross *et al*[42], 2015/Linagliptin/metformin *vs* linagliptin monotherapy	Change from baseline in HbA1c	Randomized, double-blind, active-controlled, parallel group, multinational	Newly diagnosed (≤ 12 mo) T2D and marked hyperglycemia (≥ 8.5 and ≤ 12.0%)	ACS, stroke or TIA < 3 mo	316	24 wk	Safety endpoint (AE)	Severe hypoglycemia: Requiring assistance from another person to administer carbohydrate or other resuscitative action	Linagliptin/metformin: 1.9% of patients and linagliptin: 3.2% of patients no severe hypoglycemia	-	-	No deaths

ACS: Acute coronary syndrome; AE: Adverse event; CVD: Cardiovascular disease; DINAMIC: Diamicron MR in NIDDM: Assessing Management and Improving Control; HbA1c: Glycosylated hemoglobin; HE: Hypoglycemic event; MACE: Major adverse cardiovascular event; SMBG: Self-monitoring of blood glucose; T2D: Type 2 diabetes; TIA: Transient ischemic attack.

linagliptin group. No severe HEs was reported[42]. At week 24, there was a significant reduction in HbA1c from baseline in the linagliptin/metformin *vs* linagliptin group ($P < 0.0001$ for treatment difference)[42]. Target HbA1c of < 7.0% was achieved by 61% of patients in the linagliptin/metformin arm and 40% of patients in the linagliptin arm[42].

Other studies of linagliptin + metformin[43-45] compared the combination with either metformin or with placebo and hence were not included.

Landmark trials not meeting inclusion criteria but requiring special mention

Some landmark and important gliclazide and linagliptin trials were excluded from the narrative synthesis due to the applied exclusion criteria. However, given their importance in the drug trajectory, they require a special mention to obtain a clear picture regarding the HE and MACE AEs associated with gliclazide and linagliptin.

Excluded gliclazide trials

Action in diabetes and vascular disease, Preterax and Diamicron Modified Release Controlled Evaluation trial: Gliclazide studies retrieved during the literature search that reported MACE as an outcome were the "Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation" (ADVANCE)[23] trial and its analyses[46-53]. However, the ADVANCE trial and its analyses were excluded from the narrative synthesis because the ADVANCE trial compared high intensity glucose control (with gliclazide) with standard glucose control (with other SUs). Also, in the high intensity group, those not achieving the targeted HbA1c with highest gliclazide dose were further given metformin, thiazolidinediones, acarbose or insulin as add-on therapy[23]. Comparison studies of gliclazide *vs* other GLDs (except DPP4 inhibitors) and studies analyzing gliclazide in combinations with other GLDs (except metformin) were excluded from the analysis.

Additionally, the ADVANCE trial recruited patients at high CV risk[23,54]. Patients with a history of stroke, MI, unstable angina, transient ischemic attack and coronary or peripheral vascularization met the inclusion criteria for the study[23,54]. Thus, the ADVANCE trial evaluated the MACE outcome in patients at high risk for MACE. However, the ADVANCE trial also recruited patients with no history of CVD but at high risk of MACE as they had T2D for ≥ 10 years or were ≥ 65-years-old.

The primary macrovascular endpoint of the ADVANCE trial was a composite of CV endpoints (death from CV causes, nonfatal MI or nonfatal stroke). Individual CV endpoints were evaluated as secondary endpoints[23,54]. The trial also evaluated microvascular endpoints both as a composite and individual endpoint[23,54]. During the 5-year follow-up there were no significant effects of the type of glucose control on major macrovascular events[23].

Hypoglycemia was a secondary endpoint of the ADVANCE trial. It was defined as a BG level of < 2.8 mmol/L (< 50.5 mg/dL) or the presence of typical symptoms and signs of hypoglycemia without other apparent causes. Patients with transient dysfunction of the central nervous system requiring external help for treatment were considered to have severe hypoglycemia. During the 5-year follow-up severe hypoglycemia was uncommon. However, it was significantly more common in the intensive-control than standard-control group (2.7% *vs* 1.5%)[23].

Excluded linagliptin trials

Cardiovascular and Renal Microvascular Outcome Study With Linagliptin trial: The other study of linagliptin *vs* placebo that reported both HE and MACE as outcomes was the landmark "Cardiovascular and Renal Microvascular Outcome Study With Linagliptin" (CARMELINA) trial. This study was excluded from the narrative synthesis because it evaluated HE and MACE in 6979 T2D patients with high CV and renal risk[8]. However, given that this was a landmark trial, it is discussed in the excluded linagliptin studies section.

This study evaluated HE and MACE in 6979 T2D patients with high CV and renal risk[8]. The trial, designed as a non-inferiority trial of linagliptin *vs* placebo, assessed the first occurrence of the composite of MACE as a primary endpoint and hypoglycemia was assessed as an AE. The outcomes of interest were well defined according to predefined criteria. After a median follow-up of 2.2 years, MACE occurred in 12.4% and 12.1% in the linagliptin and placebo groups, respectively, and the difference was statistically significant[8]. The frequency of confirmed HEs including severe hypoglycemia in the linagliptin *vs* placebo group was 15.9% *vs* 16.4%. HE in the placebo group was due to rescue medications that were allowed to control hyperglycemia[8].

CAROLINA trial: In the CAROLINA trial, 6042 subjects with T2D and HbA1c of 6.5%-8.5% who were at high CV risk (had established CV disease and renal impairment but not end stage renal disease) were randomized to linagliptin at 5 mg/d (n = 3028) *vs* glimepiride at doses of 1-4 mg/d (n = 3014)[9]. After a mean follow-up of 6.3 years, the primary outcome of the trial (MACE) occurred in 11.8% of subjects in the linagliptin arm *vs* 12% of subjects in the glimepiride arm, and the difference was statistically significant[9]. At least one HE occurred in 10.6% *vs* 37.7% of participants in the linagliptin *vs* glimepiride group, respectively[9].

DISCUSSION

There were no CVOT trials for gliclazide. The landmark ADVANCE trial[23] compared two levels of glycemic control, intensive (HbA1c < 6.5%) *vs* standard (managed with oral GLD according to local practice). It was not a CV safety trial of gliclazide, but the trial did show that the primary endpoint of the composite of microvascular and macrovascular events was significantly reduced by 18.1% in the

intensive control gliclazide arm.

On the other hand, CV safety of linagliptin has been demonstrated by two RCTs, namely the CARMELINA[8] (*vs* placebo) and the CAROLINA[9] (*vs* glimepiride, a SU) trials. These dual randomized CVOT linagliptin trials in T2D patients (CARMELINA[8] and CAROLINA[9]) showed that linagliptin was non-inferior to placebo and glimepiride, respectively, for the composite of MACE.

This CV safety of gliclazide in the ADVANCE trial and of linagliptin in the CARMELINA and CAROLINA trials was demonstrated in patients at high risk of CVD. Hence, gliclazide and linagliptin can be considered as oral GLD that can be given safely in T2D patients with CVD or at high risk of CVD.

In this context, the two RCTs comparing gliclazide with vildagliptin, a DPP4 inhibitor[34,35], were not powered to assess hypoglycemia and MACE as outcomes. Instead, they reported these as AEs. However, neither trial reported a significant difference in CV safety and/or HE incidence between gliclazide and vildagliptin. In this context, it is important to note that linagliptin and vildagliptin belong to two different classes of DPP4 inhibitors[55]. Hence, it is important to compare gliclazide with linagliptin.

Also, all SUs do not have the same CV risks. SUs like glyburide/glibenclamide inhibit an ATP-sensitive potassium channel in the heart and pancreas and are therefore associated with increased CV risk as compared to gliclazide, which selectively inhibits ATP-sensitive potassium channels only in the pancreas[56]. The CARMELINA trial compared linagliptin with glimepiride. However, the double-blind head-to-head comparison GUIDE study showed that compared to glimepiride, gliclazide had a better safety profile and resulted in 50% fewer HEs[2]. The frequency of CV AEs was similar in both glimepiride and gliclazide groups and judged by the investigator as not related to the treatment[2].

Strengths and limitations

Literature was searched using only free resources such as MEDLINE and Google scholar. Hence, the SR is likely to have missed some important articles on the paid sites. The strict inclusion and exclusion criteria is likely to have filtered out important RCTs and real-world studies that could have added value to the CV and hypoglycemia profile of these two drugs. This SR was also limited by its reporting style of narrative synthesis. However, as explained under the "Narrative synthesis of data" section, there were no trials comparing gliclazide and linagliptin. Hence, gliclazide and linagliptin studies were independently assessed for the outcomes of interest. For most studies included in the narrative synthesis, except the CARMELINA[8], ADVANCE[23] and Diamicron MR in NIDDM: Assessing Management and Improving Control 1 study[33], hypoglycemia, MI and other CV events were reported as cause of exclusion from the study or withdrawal from study and non-inclusion in analysis. Hence, these trials looked at outcome of interest in patients, not at risk of CV and renal events.

Filtering of gliclazide trials by the year (2008) resulted in inclusion of trials in the later trajectory of gliclazide compared to linagliptin trials that were in the earlier stage of drug trajectory. This resulted in exclusion of five randomized gliclazide clinical trials that reported the outcomes of interest in the initial drug trajectory[2,57-60] These included trials compared various gliclazide formulations[57,60] and trials comparing gliclazide with other SUs such as the GUIDE Study[2] and with thiazolidinediones (QUARTET Study Group)[58]. However, none of these RCTs included a DPP4 inhibitor as a comparator. Hence, their exclusion did not affect the narrative synthesis.

All the records included in this study were RCTs or a factorial randomized design. Hence, quality of records included was good.

CONCLUSION

Although, the head-to-head comparative clinical data between gliclazide and linagliptin is lacking, both the drugs have shown effective glycemic control along with CV safety in patients with T2D. In resource limited settings, SUs are commonly used as the first add-on therapy after metformin because of cost constraints. In these settings, there is a need to compare modern Sus like gliclazide, which have a cardiac-sparing action, with drugs with established CV safety in CVOT such as DPP4 inhibitors. Future RCTs may confirm the comparative CV outcomes between gliclazide and linagliptin and other DPP4 inhibitors.

ARTICLE HIGHLIGHTS

Research background

Type 2 diabetes (T2D) patients are at increased cardiovascular and treatment-related hypoglycemia risk. Various guidelines recommend dipeptidyl peptidase-4 (DPP4) inhibitors as the first add-on therapy to metformin in T2D due to their confirmed cardiovascular benefits demonstrated through cardiovascular outcome trials. However, in resource limited countries like India, newer sulfonylureas, like gliclazide and glimepiride, are the most commonly used glucose-lowering drugs in T2D due to their low cost.

Gliclazide and glimepiride have similar glycemic efficacy, but gliclazide has a 50% lower hypoglycemia risk.

Research motivation

A landmark cardiovascular outcome trial demonstrated the cardiovascular safety of glimepiride against linagliptin (a DPP4 inhibitor). However, the cardiovascular safety of gliclazide *vs* linagliptin has not been established through cardiovascular outcome trials. If the cardiovascular safety and lower hypoglycemia risk of gliclazide is established *vs* linagliptin, it will help physicians prescribe it with assurance of safety for their patients.

Research objectives

To assess the cardiovascular safety and hypoglycemia risk of gliclazide as compared to linagliptin (and other DPP4 inhibitors). The objective was to assess whether gliclazide was as safe as the guideline recommended DPP4 inhibitor (linagliptin) in providing cardiovascular safety and lowering hypoglycemia risk in T2D. This systematic review was likely to help provide assurance regarding cardiovascular and hypoglycemia safety of gliclazide in T2D as compared to costlier DPP4 inhibitors.

Research methods

This systematic review followed the current Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines to analyze all the clinical studies published from 2008 through the present that compared the cardiovascular safety and hypoglycemia risk of the two drugs in patients with T2D with no cardiovascular disease. Using keywords such as “linagliptin”, “Gliclazide”, “hypoglycemia”, “myocardial infarction”, and “cardiovascular death”, we searched the databases MEDLINE and Google Scholar. Two independent reviewers assessed the trials included using the current Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines for systematic reviews. We included only evidence designated high quality by the Oxford Center for Evidence-based Medicine-Levels of Evidence. The primary outcomes compared were major adverse cardiovascular events and hypoglycemia risk.

Research results

We could not find any trial comparing gliclazide with linagliptin, either as monotherapy or as add-on therapy to metformin. The cardiovascular safety of gliclazide in the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial and of linagliptin in the Cardiovascular and Renal Microvascular Outcome Study With Linagliptin (CARMELINA) and CARdiovascular Outcome study of LINAgliptin *vs* glimepiride in patients with T2D (CAROLINA) trials were excluded from the comparative analysis as these trials demonstrated cardiovascular and hypoglycemia benefits in patients at high risk of cardiovascular disease. However, since these are landmark trials, their results are important and hence described in detail as a separate section. The final analysis included five gliclazide and three linagliptin trials (total eight studies) that individually studied the outcomes of interest in T2D patients with no established cardiovascular disease. Statistical comparisons of the results were not possible as the trials had different designs, different definitions of major adverse cardiovascular events and hypoglycemia and were conducted in different patient populations. Hence, no direct comparisons were possible. The trials were therefore described individually, and their results were compared through narrative synthesis. We assessed that both drugs were effective in achieving the desired glycemic control and had low major adverse cardiovascular events and hypoglycemia risk in adult patients with no cardiovascular disease.

Research conclusions

Gliclazide can be considered as an effective and safe glucose-lowering drug in T2D patients with no established cardiovascular disease but at high risk of cardiovascular disease due to their T2D status.

Research perspectives

Future randomized controlled trials comparing gliclazide with linagliptin or DPP4 inhibitors can add value to the findings of this systematic review.

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FOOTNOTES

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