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EDITORIAL

Receptor activator of nuclear factor κB ligand/osteoprotegerin axis and vascular calcifications in patients with chronic kidney disease

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Abstract

Vascular calcifications are commonly observed in patients with chronic kidney disease (CKD) and contribute to the excessive cardiovascular morbidity and mortality rates observed in these patients populations. Although the pathogenetic mechanisms are not yet fully elucidated, recent evidence suggests a link between bone metabolism and the development and progression of vascular calcifications. Moreover, accumulating data indicate that receptor activator of nuclear factor κB ligand/osteoprotegerin axis which plays essential roles in the regulation of bone metabolism is also involved in extra-osseous bone formation. Further studies are required to establish the prognostic significance of the above biomarkers as predictors of the presence and severity of vascular calcifications in CKD patients and of cardiovascular morbidity and mortality. Moreover, randomized clinical trials are needed to clarify whether inhibition of osteoclast activity will protect from vascular calcifications.

Key words: Arterial stiffness; Bone turnover; Chronic kidney disease; Osteoprotegerin; RANK ligand; Receptor activator nuclear factor κB; Vascular calcifications

Core tip: Vascular calcifications are commonly observed in chronic kidney disease patients and recently mounting evidence suggest that Receptor activator of nuclear factor κB ligand/osteoprotegerin axis controls both bone metabolism and extra-osseous bone formation. Further studies are required to establish the role of these biomarkers as predictors of the presence and severity of vascular calcifications and of cardiovascular morbidity and mortality. Moreover, randomized clinical trials are needed to clarify whether inhibition of osteoclast activity will protect from vascular calcifications.

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INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of excessive morbidity and mortality for patients with chronic kidney disease (CKD) and particularly those with end-stage renal disease (ESRD) on renal replacement therapy with either hemodialysis or peritoneal dialysis[1]. Vascular calcifications are also commonly observed in CKD and are now considered part of the syndrome chronic kidney disease-mineral and bone disorder (CKD-MBD), the pathogenesis of which has accumulated great research interest the last years. In CKD patients calcifications in both intimal (atherosclerotic) and medial lamina (arteriosclerotic) often coexist, appear early and follow an accelerated course. Particularly the latter is an almost ubiquitous feature of arterial tree in chronic uremia and a major contributor to the accelerated arteriosclerosis and to the increased all-cause and CVD mortality in these patients populations[2].

The presence of vascular calcifications in CKD has been associated with a number of traditional risk factors including older age, hypertension, dyslipidemia and diabetes mellitus which are highly prevalent in this population, as well as uremia-related risk factors including chronic inflammation, oxidative stress and mineral and bone disorders which are currently under investigation. Of note, mineral alterations (hypercalcemia, hyperphosphatemia) and disorders of bone metabolism (both secondary hyperparathyroidism and adynamic bone disease) are mainly associated with the development and progression of medial but not intimal calcifications[3].

It is now well recognized that vascular calcification is not simply a passive physicochemical process of calcium phosphate deposition but a highly regulated active process similar to normal bone modeling. Moreover, recent evidence suggests that the phenotypic transdifferentiation of vascular smooth muscle cells (VSMCs) into osteoblast-like cells is a key pathogenetic event in the osteogenesis of the vascular wall. A variety of regulatory factors and molecular pathways of this process which regulate bone turnover and/or mineralization have been identified[3]. Although their relative importance in different disease states appear still incompletely understood emerging evidence suggest that the receptor activator of nuclear factor κB (RANK)/RANK Ligand (RANKL)/osteoprotegerin (OPG) system that plays essential roles in the regulation of bone metabolism is also involved in extra-osseous bone formation[4].

RANK/RANKL/OPG PATHWAY

RANK, RANKL and OPG are members of the tumor necrosis factor (TNF) superfamily which were originally studied as factors involved in bone tissue and immune system physiology. However, recent studies revealed that they also constitute a link between bone metabolism and vascular pathophysiology by controlling simultaneously bone remodeling and vascular calcification mechanisms[5].

RANK/RANKL/OPG signaling pathway regulates osteoclast differentiation and activation. RANKL is a transmembrane protein consisted of 316 aminoacids, which is expressed on osteoblasts, stromal and T cells in areas of bone remodeling. RANKL binds to its receptor RANK, a 616 amino acid type Ⅰ transmembrane protein which is expressed on the surface of myeloid cell lineages like osteoclasts, monocytic and dendritic cells. The above activates multiple intracellular signals, including activation of the c-jun N-terminal kinase and nuclear factor κB pathways that regulate the differentiation, function and survival of these cells. A variety of factors including hormones, cytokines and growth factors regulate its expression. Thus, parathormone (PTH), TNF-α, calcium, corticosteroids and several interleukins (IL-6,-11,-17) increase RANKL expression on osteoblasts, whereas transforming growth factor-β (TGF-β) decreases it[3,5]. OPG is a soluble glycoprotein widely expressed in most human tissues including bone (osteoblests, mesenchymal stem cells), immune cells (T and B cells) and vessels (endothelial and VSMCs). It acts as a decoy receptor and binds to RANKL, thereby not allowing the activation of RANK and inhibits its regulatory effects on inflammation, skeletal and vascular systems[5]. OPG also has anti-apoptotic actions, as it binds and deactivates the TNF related apoptosis-inducing ligand (TRAIL), which is expressed by many cell types, including VSMCs, and can also lead to ectopic mineralization[5]. OPG's expression is increased by vitamin D, TNF-α, IL-1α, IL-6, IL-11 and IL-17, bone morphogenic protein-2, TGF-β and estrogens, whereas it is reduced by PTH, corticosteroids and prostaglandin E2[5].

Mounting evidence suggests that the RANK/RANKL/OPG axis exerts actions simultaneously on endothelial cells and VSMCs and participate in multiple processes that regulate vascular calcification. The implication of this system in vascular pathophysiology is supported by the expression of these molecules in the normal cardiovascular system (heart, arteries and veins). Both endothelial cells and VSMCs constitutively express OPG, and their levels are particularly high in aortic and renal arteries. Furthermore, OPG is physically associated with factor Ⅷ-von Willebrand factor complex localized in the Weibel–Palade bodies of endothelial cells, it is rapidly secreted in response to inflammatory stimuli and inhibits osteoclastogenesis and promotes endothelial cell survival[6] through neutralization of pro-apoptotic TRAIL[5]. In contrast, RANKL and RANK are frequently undetectable in normal vessels and non-calcified arteries or valves[5]. However, osteoclast-like RANK(+) cells were found close to VSMCs on calcified vascular walls and

expression of both RANK and RANKL was reported on the vascular wall, in calcified areas[7].

Several studies suggested that OPG's expression might reflect a protective mechanism against the vascular calcifications. Thus, over-expression of OPG leads to osteopetrosis, while its gene deletion, increases bone metabolism and leads to osteoporosis and medial calcifications in the aorta and the renal arteries[8,9]. Moreover, OPG administration in laboratory animals, was reported to potently reduce both bone resorption activity and medial arterial calcifications induced by administration of toxic doses of vitamin D or warfarin[10]. These findings, together with the fact that OPG is expressed in the vascular wall under normal circumstances, indicate that the endogenous production of OPG prevents the ossification of the vascular wall and favors bone mineralization. In accordance with the above, OPG was detected in matrix vesicles, nanoparticles that are released from VSMCs with the capacity to nucleate mineral, which directly inhibited deposition of hydroxyapatite in the vascular wall[11].

OPG AND VASCULAR CALCIFICATIONS IN CKD PATIENTS

In the general population, studies have demonstrated that high levels of OPG are correlated with cardiovascular risk[12]. In CKD patients, it has been shown that OPG levels significantly increase along with the decline in GFR and are reduced after a successful renal transplantation[13]. However, studies examining the association of OPG levels with the presence and extent of cardiovascular calcifications are relatively limited and their results were sometimes inconsistent. Thus, in non-dialyzed CKD patients a cut-off value of OPG level was found to predict the presence of coronary artery calcifications (CAC) assessed by chest multidetector computed tomography[14]. In addition, a very recent study in CKD patients reported a significant association between high OPG levels and CAC independently of other risk factors including age, gender, diabetes, body mass index and smoking habits[15]. In transplanted patients CAC at baseline, but not 1 year after renal transplantation, was found to be independently associated with baseline OPG whereas post-transplant CAC progression was predicted by baseline CAC score[16]. In contrast, another study in transplanted patients reported that OPG levels were significantly and independently associated with the progression of aortic calcification index (ACI) assessed by lateral lumbar x-ray during a two-year follow-up period[17]. In adults and children with ESRD on hemodialysis, studies demonstrated a significant independent correlation between OPG levels and CAC[18,19]. Similarly, another study showed an association between OPG and ACI assessed by computed tomography scans independently of traditional and uremia-related risk factors[20]. In addition, high OPG levels have been found to correlate with faster progression of aortic calcifications during a 5-year follow-up[21]. Finally, several studies in patients with various CKD stages as well as in renal transplant recipients demonstrated that OPG levels were a significant independent predictor of all-cause and cardiovascular mortality during the follow-up period[22-26]. However, one study in ESRD and pre-dialysis CKD patients showed that renal function rather than OPG levels were mostly associated with the progression of aortic and coronary calcifications[27], whereas another one correlated elevated OPG levels only with moderate CAC[28].

Regarding the association of OPG with markers of medial calcifications such as arterial stiffness and pulse wave velocity (PWV), the results are also sometimes controversial. A study in non-dialyzed CKD patients demonstrated a strong relationship between serum OPG and arterial stiffness independent of many potential confounders including traditional cardiovascular risk factors, abnormal bone and mineral metabolism, and inflammation[29]. Similarly, in hemodialysis patients OPG levels were found to be strongly associated with aortic or carotid-to-femoral PWV independently of traditional and uremia-related risk factors including markers of inflammation[25,26,30]. However, other studies in adults and children on hemodialysis treatment were unable to confirm the above findings[19,31].

As it was previously noted, the exact role of OPG in the VC process remains unclear. Since OPG inhibits osteoclast activity and OPG knockout mice develop arterial calcifications, it appears reasonable to assume that it plays some regulatory and/or inhibitory role against ectopic calcifications and thus its increased levels could be interpreted as an attempt to compensate for the ongoing calcification process[9-11]. The above speculation contradicts the reported association of high OPG levels with cardiovascular mortality[22,23,25,26] and moreover, increase of OPG levels could be due to its production by calcified vascular cells in conditions of diffuse calcification. Thus, it remains to be clarified whether the elevated OPG levels induce arterial wall sclerosis or represent a compensatory mechanism to prevent further arterial damage or are just a marker of initiation of vascular calcification process[4].

RANKL AND VASCULAR CALCIFICATIONS IN CKD PATIENTS

The exact role of RANKL in the development of cardiovascular calcifications and CVD remains to be identified. Some studies showed a correlation of RANKL levels with future cardiovascular events[32], but the probable association between vascular calcifications and RANKL levels has been scarcely investigated so far. A prospective study in 3250 Framingham Study participants reported that RANKL concentrations were inversely associated with multiple cardiovascular risk factors, including smoking, diabetes, and antihypertensive treatment, but that were not related with CAC or incident CVD or mortality during

a mean follow-up of 4.6 years[33]. However, a study in hemodialysis patients found that change in OPG levels after 1-year were an independent predictor of CAC score progression during the same period[34]. Of note, RANKL levels, in contrast with OPG, are low in CKD patients[35]. Considering the fact that OPG and RANKL have opposite functions in bone resorption, OPG/RANKL ratio could be considered a better biomarker of bone metabolism and consequently a better predictor of the presence and severity of vascular calcifications[35]. In agreement with the above hypothesis, the aforementioned study in hemodialysis patients, showed that baseline OPG/RANKL ratio was significantly higher in patients whose coronary calcifications progressed during the one year follow up period[34].

CONCLUSION

Despite the progress and the knowledge acquired within the previous years, the pathogenesis of vascular calcifications remains to be fully elucidated. Recently, mounting evidence suggest that RANKL/RANK/OPG system which controls bone metabolism plays a significant role in this process. Alterations of RANKL/OPG axis appear a promising prognostic biomarker of the initiation and progression of vascular calcifications in CKD patients and of cardiovascular morbidity and mortality. Further studies are required to establish this theory and to identify the exact role of these two biomarkers in CKD patients. Moreover, randomized clinical trials are needed to clarify whether inhibition of osteoclast activity will protect from vascular calcifications.

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FRONTIER

Update on immunoglobulin a nephropathy. Part Ⅱ: Clinical, diagnostic and therapeutical aspects

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Abstract

Immunoglobulin A nephropathy (IgAN) is characterized by different clinical manifestations and by long-term different outcomes. Major problem for the physicians is to understanding which patients are at risk of a disease evolution and to prescribe the right therapy to the right patients. Indeed, in addition to patients with a stable disease with no trend to evolution or even with a spontaneous recovery, patients with an active disease and patients with a rapidly evolving glomerulonephritis are described. Several histopathological, biological and clinical markers have been described and are currently used to a better understanding of patients at risk, to suggest the right therapy and to monitor the therapy effect and the IgAN evolution over time. The clinical markers are the most reliable and allow to divide the IgAN patients into three categories: The low risk patients, the intermediate risk patients and the high risk patients. Accordingly, the therapeutic measures range from no therapy with the only need of repeated controls, to supportive therapy eventually associated with low dose immunosuppression, to immunosuppressive treatment in the attempt to avoid the evolution to end stage renal disease. However the current evidence about the different therapies is still matter of discussion. New drugs are in the pipeline and are described. They are object of randomized controlled trials, but studies with a number of patients adequately powered and with a long follow up are needed to evaluate efficacy and safety of these new drugs.

Key words: IgA nephropathy prevention and control; IgA nephropathy; IgA nephropathy diagnosis; IgA nephropathy prognosis; IgA nephropathy classification; IgA nephropathy therapy

Core tip: Primary immunoglobulin A nephropathy (IgAN) is the most frequent glomerulonephritis. The IgAN is a relatively benign disease however, the long term prognosis should not be considered mild, because, after

20 years of disease evolution, 25% of the patients are going into chronic renal failure. It is essential to find out the risk factors predicting the evolution to end-stage renal disease (ESRD) and to select those patients who may benefit from immunosuppressive treatment. For all patients, it is essential to have a regular clinical control to check any disease evolution, in order to avoid or delay the disease progression to ESRD.

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INTRODUCTION

Previously[1] we highlighted that, the diagnosis of immunoglobulin A nephropathy (IgAN) is principally based on a renal biopsy because there are different histological image results and different clinical presentation.

As the clinical presentations may be extremely different a tentative clinical classification aimed for the best therapy may be particularly useful.

Floege *et al*[2] categorized the clinical scenarios of IgAN into four classes: (1) patients diagnosed accidentally while looking for clinical manifestations such as reduced glomerular filtration rate (GFR), hypertension and urinary abnormalities. Such patients may be affected by IgAN and are called the silent majority; (2) patients affected by recurrent macroscopic hematuria strictly connected with acute infective diseases occurring in the upper respiratory tract. Such patients are also called typical IgAN patients and, in addition to hematuria, may be affected by proteinuria, hypertension and reduced GFR, which represent signs of disease evolution[3]; (3) patients presenting atypical signs such as nephritic syndrome and acute or rapidly progressive renal disease; and (4) patients with IgAN recurrence after kidney transplantation.

In addition to these categories, there are patients presenting acute kidney injury (AKI) accompanying macroscopic hematuria, due to acute tubular necrosis and intratubular erythrocyte casts. The relevance of this IgAN presentation is represented by the good prognosis not characterized by a disease progression.

Finally, IgAN may affect subjects who are otherwise healthy as documented by biopsies of kidneys suitable for transplantation or by autopsies of subjects not affected by renal diseases[4,5].

A further difficulty in decision making on therapeutic approaches is related to the fact that most subjects with IgAN may have a benign course or even disease resolution as documented by cohorts of patients followed for 10 years after diagnosis in China and Spain[6,7].

Such extremely variable clinical presentations and disease evolution have two principal consequences: (1) most guidelines concerning IgAN, such as the Kidney Disease Improving Global Outcomes (KDIGO)[8], are based on a low level of evidence and are often based on opinions. As a consequence, there are few guidelines based on recommendations and the majority are only suggestions. Indeed, whether to treat and the beneficial effects of many treatments remain to be better validated[9]; and (2) There is a need for research on histological, biological and clinical markers that are able to predict the risk of IgAN progression and to guide therapeutic decisions and monitor therapeutic results.

Indeed, only a fraction of IgAN patients require treatment to prevent disease progression, and predicting which patients are at risk of progression is of overwhelming importance. Different histological, biological and clinical markers of prognosis have been identified, and other markers will likely be validated[10].

RESEARCH METHODOLOGY

We have analyzed the available papers on IgAN diagnosis, IgAN prognosis and IgAN therapy by a review of the currently available papers. A literature search was performed using PubMed (NCBI/NIH) with the search words "IgAN diagnosis", "IgAN prognosis", "IgAN biomarkers", "IgAN classification", and IgAN therapy". As first line research the papers published in the last three years were examined. Paper selection has been mede according the relevance of the journal, the authors, the dimension of the study and the novelty of the findings. So doing 40 papers recently published have been selected, then we proceeded in a backward way and studies previously published have also been included. Studies currently under way were searched for in "clinical trial.gov" and the European EUDRACT register. As clinical trial.gov also includes studies that to date are either closed or have not started, we selected only randomized controlled trials (RCTs) that are active and enrolling patients. So doing we report 15 RCTs out of the 68 that may be found on clinical.trial.gov. The RCTs exluded are either terminated or closed or not enrolling patients.

DIAGNOSIS AND PROGNOSIS

Histological markers

The glomerular histopathology in the IgAN is extremely variable, and its identification and reproducibility among different observers is essential to establish any relationship between renal pathology and disease evolution[11].

The glomerular abnormalities range from minimal abnormalities to mesangial hypercellularity, endocapillary hypercellularity, extra capillary hypercellularity, and segmental glomerulosclerosis.

The tubulointerstitial lesions may be near normal, but in some patients a tubular injury resulting in a fibro proliferative peritubular response is observed. In addition, several clinicopathological correlations have reported that the tubular atrophy is the most reliable

Table 1 Definitions of pathological variables used in the Oxford classification of immunoglobulin a nephropathy

Variable	Definition	Score
Mesangial hypercellularity	< 4 Mesangial cells/mesangial area = 0 4-5 Mesangial cells/mesangial area = 1 6-7 Mesangial cells/mesangial area = 2 > 8 Mesangial cells/mesangial area = 3	M0 < 0.5 M1 > 0.5
Segmental glomerulosclerosis	Any amount of the tuft involved in sclerosis, but not involving the whole tuft or the presence of an adhesion	S0 = absent S1 = present
Endocapillary hypercellularity	Hypercellularity due to increased number of cells within glomerular capillary lumina causing narrowing of the lumina	E0 = absent E1 = present
Tubular atrophy/ interstitial fibrosis	Percentage of cortical area involved by the tubular atrophy or interstitial fibrosis, whichever is greater	0%-25% - T0 26%-50% - T1 > 50% - T2

Table 2 Summary of studies correlating the Oxford classification for immunoglobulin a nephropathy with clinical outcomes

Study	Patients (*n*)	End point	Univariate analysis	Multivariate analysis
Coppo *et al*[19]	206 A, 59 C	Rate of eGFR decline	M, E, S T	M, E, S, T
Herzenberg *et al*[20]	143 A, 44 C	Rate of eGFR decline	Not done	E, S, T
Katafuchi *et al*[21]	702 A, C	ESRD	Not done	S, T
Zeng *et al*[22]	1026 A	Rate of eGFR decline	M, S, T	M, T
Shi *et al*[23]	410 A	ESRD	M, S, T	S, T
Edström Halling *et al*[24]	99 C	GFR reduction > 50%, ESRD	M, E, T	E
Shima *et al*[25]	161 C	eGFR < 60% mL/min per 1.73m^2	M, T	M, T
Coppo *et al*[26]	973 A, 174 C	Rate of eGFR decline	M, E, S, T	S, T
Alamartine *et al*[27]	183 A	Doubling of SCr or ESRD	E, S, T	None
El Karoui *et al*[28]	128 A	Rate of eGFR decline	Not done	T
Lee *et al*[29]	69 A	GFR reduction > 50%, ESRD	E, T	E
Kang *et al*[30]	197 A	GFR reduction > 50%, ESRD	T	T
Le *et al*[31]	218 C	eGFR reduction > 50%, ESRD	T, S	T

A: Adults; C: Children; eGFR: Estimated glomerular filtration rate; E: Endothelial hypercellularity; ESRD: End-stage renal disease; GFR: Glomerular filtration rate; M: Mesangial hypercellularity; S: Segmental sclerosis; Scr: Serum creatinine; T: Tubular atrophy/interstitial fibrosis.

marker of an adverse outcome[11].

Several histological classifications have been proposed in an attempt to provide a valuable grading of histological damage and a clinico-pathological relationship. Until recently, the classifications from Lee *et al*[12], Haas *et al*[13] and Wakai *et al*[14] has been used the most. All of these classifications have the weakness of not distinguishing between the histological markers of acute activity and chronic activity of the disease. As a consequence, they fail to provide useful information concerning therapy for the acute and evolving phase of the disease.

Later on, an international working group of over 40 pathologists and nephrologists developed an evidence-based and reproducible classification for IgAN[15]. Data were obtained from 265 patients affected by IgAN who were followed for 5 years. Four histological variables had an independent value in predicting renal outcomes: Mesangial hypercellularity scores (M), segmental glomerulosclerosis (S), endocapillary hypercellularity (E) and tubular atrophy/interstitial fibrosis (T). This study led to the formulation of the Oxford classification (Table 1).

The Oxford classification has some limitations that should be remarked as the authors themselves recognize. The study is retrospective and the material comes from different countries and different centers, each with a specific and different method of evaluating renal function. In addition, the median number of glomeruli with crescents was only 9% and no patient had more than 55% of glomeruli with crescents. As a consequence, as remarked by other studies[16] in this cohort the prognostic significance of crescents is poor. Other limitations of the Oxford classification is the lack of immunohistochemical findings as the authors recognize in a further study[17]. This lack in addition to other points claims for the need of more validation studies[18].

Indeed, the prognostic value of the Oxford classification required validation and, since the Oxford classification was published, at least 17 validation studies have been reported. Eight of these studies were able to validate the classification (Table 2), principally highlighting the relevance of T, S and M scores[19-26]. Five more studies apparently did not validate completely the Oxford classification[27-31].

Validation of the Oxford classification of the IgA (VALIGA) is one of the more recent validation studies[26]. This study involved 1147 patients from 13 European countries. The principal conclusions of the study were that M, S and T lesions independently predicted eGFR loss and lower survival rates, but the addition of M, S

Table 3 Potential biomarkers for immunoglobulin a nephropathy

Biologics	Source	Rationale
Galactose deficient IgA1	Serum	Core antigen of the pathogenic IgA1 immune complex; leads to activation of mesangial cells and glomerulonephritis
Glycan-specific IgG	Serum	Form glycan-dependent complex with galactose-deficient IgA1; alanine to serine substitution in complementary-determining region 3 of IgG heavy chain; able to differentiate IgA nephropathy patients from controls with 88% specificity and 95% sensitivity
Activated complement C3	Serum	Up-regulated level in 30% of patients; correlated with deteriorating renal function
FGF 23	Serum	FGF23 serum levels are significantly associated with IgAN progression
Soluble CD89	Serum	Low levels in patients with disease progression compared with those without disease progression
Mannose-binding lectin	Urine	Significantly higher in patients than healthy controls; associated with histopathologic aggravations such as mesangial hypercellularity, tubular atrophy, interstitial fibrosis
EGF and MCP-1	Urine	An EGF/MCP-1 ratio greater than 366.66 extends renal survival to at least 84 mo in a cohort of 44 patients
Proteomic pattern	Urine	High throughput characterization of 2000 polypeptide using capillary electrophoresis on-line coupled to a mass spectrometer
microRNA profile	Urine	Sequencing identified microRNA profiling that is specific to IgA nephropathy

IgA1: Immunoglobulin A1; IgG: Immunoglobulin G; FGF23: Fibroblast growth factor 23; IgAN: Immunoglobulin a nephritis; RNA: Ribonucleic acid; EGF: Epidermal growth factor; MCP-1: Monocyte chemotactic peptide-1.

and T lesions to clinical variables predicted progression only in patients not receiving immunosuppressive treatment.

Overall, although the studies to validate the Oxford classification system led to divergent findings, this classification offers physicians a simple tool to distinguish between active and chronic lesions[32] and is the only classification system created in a truly evidence-based manner[33]. In addition, the Oxford classification system should be considered a working classification, and meetings are been held to clarify the discrepancies among the different validation studies. Waiting for further results and clarifications, to date, the KDIGO guidelines[34] do not recommend the use of pathological findings to guide therapy and predict prognosis.

The addition of clinical data to the histological findings improved the ability to predict outcomes. Indeed, in a recent study[35], a new rule to predict the risk of developing ESRD in IgAN patients was developed and validated using clinical measures together with the Oxford classification.

Biological markers

Serum and urine biomarkers may be useful both for diagnostic and prognostic purposes.

Several authors[36,37] have formulated the "four hits" theory to explain the IgAN pathogenesis. Accordingly, in a four steps fashion, after an increase of galactose deficient circulating IgA1 (Gd-IgA1), there is an antibody production against these Gd-IgA1. Later on immunocomplexes are formed and may deposit in the kidney. Finally an inflammatory response is activated.

According to the four hits theory of IgAN pathogenesis, the diagnostic biomarker's usefulness decreases from hit 1 to hit 4, while, on the contrary, the prognostic value increases[38].

Table 3 summarizes the different biomarkers and their rationale in the diagnosis[39].

Serum galactose deficient immunoglobulin A1

Galactose deficient immunoglobulin A1 (Gd-IgA1) represents a core antigen of the pathogenic IgA1 immunocomplexes and leads to activation of mesangial cells. Principally, Gd-IgA1 represents a diagnostic marker. Data from studies considering Gd-IgA1 a prognostic marker are discordant. In one study, the serum levels of Gd-IgA1 were associated with disease progression[40]. In another study, the serum levels of Gd-IgA1 did not correlate with proteinuria and eGFR decline[41].

Serum anti-glycan antibodies

This biomarker correlates with the urine protein/creatinine ratio[42] and with disease progression towards ESRD[43].

Serum breakdown of complement C3 products

Complement activation is up-regulated in 50% of patients and correlates with a decrease in renal function[44-46]. Additionally preliminary studies have documented in IgAN the association of glomerular C4d deposition with serum creatinine, proteinuria and histological damage[47]

Fibroblast growth factor 23

Fibroblast growth factor 23 (FGF23) is a circulating hormone involved in phosphate homeostasis. In a recent study, FGF23 levels were significantly associated with IgAN progression[48].

CD89-IgA complexes

The deposition of CD89-IgA complexes may facilitate mesangial cell activation. A study reported that IgAN patients without disease progression had high levels of soluble CD89, whereas patients with disease progression had low levels of soluble CD89[49].

In addition to the serum biomarkers, urinary biomarkers may also be useful both in the diagnosis of IgAN and the prognosis .

The urinary mannose-binding lectin[50] is a biomarker for predicting IgAN progression. Indeed, it is associated with the worsening of histopathologic lesions such as mesangial hypercellularity, tubular atrophy and interstitial fibrosis.

In a small cohort[51], a urinary epidermal growth factor/monocyte chemotactic peptide ratio greater than 366.66 was related to an improvement in the renal survival rate over the long term.

The relevance of urine proteomics as an alternative to single biomarkers has been evaluated[52,53]. The usefulness of proteomics as a diagnostic tool has been documented, but its value as a prognostic factor remains to be evaluated.

Several studies have evaluated the role of small microRNAs in the diagnosis and the prognosis of IgAN[54].

MicroRNAs are short, noncoding RNA molecules that regulate gene expression. Micro RNAs such as 18-5 p, 29 c, 133 a, 133 b, 148 b, 185, 192 and 200 c have been documented to exert a role in the pathogenesis of IgAN. Their level in urinary excretion may be elevated in the course of the disease and may represent a useful diagnostic tool. The prognostic value remains to be evaluated, even though the relationship between the urinary levels of miRNA 146 and miRNA 155 and proteinuria and lower GFR have recently been documented[55].

Many biological markers have been described principally as a possible diagnostic tool. Some papers have also reported their usefulness in prognosis and have described their correlation with disease evolution. However, none of these approaches has been properly confirmed as a valuable predictor of clinical outcomes, and their superiority with respect to the clinical markers is still to be proven.

Clinical markers

To date, clinical prognostic markers remain as the most reliable predictors of IgAN evolution.

Principally, they include an impaired GFR, sustained hypertension and proteinuria[56,57]. Longitudinal trends in blood pressure (BP) and proteinuria are both associated with disease progression[58,59]. In a prospective study on 332 IgAN patients[59], proteinuria > 1 g/d, and hypertension > 140/90 mmHg, when associated with severe histological lesions, allowed the calculation of a risk score predicting death or ESRD 10 years to 20 years after disease onset. Another study, based on retrospective data from 600 IgAN Chinese patients[60], identified four baseline variables with an independent risk of ESRD evolution; *i.e.,* GFR, serum albumin, hemoglobin and systolic BP. Recently, looking for the IgAN outcome predictors, a study on a multiethnic United States cohort documented that the baseline eGFR was the strongest predictor of ESRD[61]. High body mass index and smoking have also been identified as predictors of poorer outcomes in IgAN[62,63]. These factors, however, are not specific for IgAN, but are common to any glomerulonephritis.

By contrast, the degree of hematuria, which is a typical manifestation of IgAN, does not have a predictive value. As already mentioned the clinical presentation with AKI accompanying macroscopic hematuria doesn't necessarily mean crescentic IgAN but may be the expression of acute tubular necrosis spontaneously resolving.

In summary, several histological, biological and clinical markers have been proposed as predictors of IgAN outcomes and, as a consequence, are useful for suggesting therapeutic measures and monitoring their effects. However, to date, neither histological nor biological markers have documented a clear superiority over the more simple clinical markers[10].

THERAPY

From a therapeutic point of view, IgAN patients at diagnosis should be divided into three groups[10] and the therapeutic approaches differ according IgAN groups. (1) low risk patients: These are subjects with normal GFR, no hypertension and minor urinary abnormalities (proteinuria < 0.5 g/d +/- isolated microhematuria). These patients do not require treatment but should be checked annually or biannually for at least 10 years. Monitoring is recommended to check any disease evolution. In the case of disease evolution, therapeutic measures should be adopted as described below; (2) intermediate risk patients have a proteinuria > 0.5-1 g/d that may be associated with hypertension and a reduced GFR. These patients should receive optimized supportive therapy and should be strictly monitored. A corticosteroids course and/or immunosuppressive treatment might be added if proteinuria increases or GFR declines; and (3) high risk patients show a rapid decrease in the GFR that may be associated with nephritic syndrome or crescentic glomerulonephritis. These findings may be already present at IgAN diagnosis or may develop during the disease evolution. In addition to supportive treatment, corticosteroids and immunosuppression should be considered for these high risk patients.

Supportive care

Supportive care is recommended by KDIGO guidelines[34] for any IgAN patient at risk of disease evolution.

The supportive care includes several measures aimed to control the progression of any glomerulonephritis, among which is IgAN (Table 4)[64].

The mainstay of supportive treatment in IgAN is the control of BP and control of the renin-angiotensin system (RAS)[65].

A review of 11 RCTs, documented that treatment with angiotensin converting enzyme inhibitors (ACEI) or with angiotensin receptor blockers (ARB) significantly reduced proteinuria and had a renoprotective effect with respect to the controls[66]. These data were confirmed by a meta-analysis that reviewed 6 RCTs[67].

Table 4 Supportive therapy of immunoglobulin a nephropathy

Level 1	Control blood pressure (sitting systolic BP in the 120 s)
	ACE inhibitor or ARB therapy with up-titration of dosage or combination ACE inhibitor and ARB therapy
Level 2	Control protein intake
	Restrict NaCl intake/institute diuretic therapy
	Control each component of the metabolic syndrome
	Aldosterone antagonist therapy
	Beta-blocker therapy
	Smoking cessation
Other measures	Allopurinol therapy
	Empiric $NaHCO_3$ therapy, independent of whether metabolic acidosis is present or not
	Avoid NSAIDs altogether, or no more than once or twice weekly at most
	Avoid prolonged severe hypokalemia
	Avoid phosphate cathartics
	Ergocalciferol therapy to correct vitamin D deficiency
	Control hyperphosphatemia and hyperparathyroidism

ACE: Angiotensin-converting enzyme; ARB: Angiotensin receptor blocker; NaCl: Sodium chloride; $NaHCO_3$: Sodium bicarbonate; NSAID: Non-steroidal anti-inflammatory drug.

More recently, the beneficial effect of Aliskiren, a direct renin inhibitor, has been documented by two studies[68,69]. Its protective effect principally is a consequence of proteinuria reduction.

In addition, a wide Cochrane review of 56 RCTs including 2838 IgAN patients[70] documented that antihypertensive agents, in particular the RAS inhibitors were more powerful renoprotective agents among the non-immunosuppressive therapies. Indeed, the effect of antihypertensive agents was compared with treatments such as fish oil supplementation, antiplatelets/anticoagulants agents and other treatments such as statins, phenytoin, herbal medicine, vitamin E and sodium cromoglycate.

Other controversial non-immunosuppressive treatments

Fish oil supplementation is an old therapy with varied results.

In a meta-analysis of fish oil therapies, no significant beneficial result was observed[71]. In the largest RCT with fish oil, an improvement in disease evolution was observed in treated patients[72], but these results were not confirmed in a more recent RCT[73].

Antiplatelet and anticoagulant based therapy is widely used in Asia. A small study documented some efficacy with dipiridamole and warfarin, but the study did not have a control group[74].

In a recent study[75] a beneficial effect was observed using statins. The study was small, not controlled, and the effect of statins on IgAN remained unclear.

In summary, as documented by the above mentioned Cochrane review[70] and after comparing the different non-immunosuppressive treatments, the only documented beneficial effect is exerted by the antihypertensive drugs, and this effect seems to be mediated by proteinuria reduction. In a recent meta-analysis[76], combination therapy with ACEI and ARB seems to achieve more benefits, even if the long-term effects still need to be documented.

Tonsillectomy

The efficacy of tonsillectomy alone or associated with immunosuppression has been a matter of discussion, and discordant results have been reported for a long time. The rationale of tonsillectomy in IgAN prevention and/or treatment is the elimination of an important source of pathogens by removing tonsil crypts. Indeed, a recent study[77] has indicated that palatine tonsils are probably a major site of Gd-IgA1 producing cells. In some patients these cells may be largely present in other lymphoid organs, and this fact might explain the diverging results of tonsillectomy.

Tonsillectomy associated with pulse steroids or other immunosuppressants is largely used in Japan, as documented by several retrospective studies[78,79]. In addition, a recent meta-analysis of seven non-randomized studies (6 in Japan and 1 in China) documented an overall beneficial effect of tonsillectomy plus corticosteroids[80]. In another meta-analysis from China, of 14 studies including 1794 patients[81], the authors concluded that tonsillectomy may induce clinical remission, but the adjustment for confounding variables could not be performed because the majority of the studies included retrospective cohorts of patients.

Recently, the first national multicenter RCT from Japan failed to demonstrate any superior effect of tonsillectomy associated with pulse steroids over pulse steroids alone[82].

Because other studies on Chinese[83] and Caucasian patients[84,85] did not confirm the tonsillectomy beneficial effect, waiting for an adequately powered RCT tonsillectomy should not be recommended. The KDIGO suggested that tonsillectomy should not be performed to treat IgAN[8]. A retrospective study on 1147 European patients with IgAN failed to demonstrate a significant correlation between tonsillectomy and renal function decline[86].

Corticosteroids

To date, the KDIGO guidelines[34] suggest a 6 mo course

of corticosteroids only for those patients at intermediate risk of having persisting proteinuria > 1 g/d and with a GFR between 30 mL/min per 1.73 m^2 and 50 mL/min per 1.73 m^2, after optimization of supportive therapy. Several studies have been performed to evaluate the usefulness of corticosteroid therapy in IgAN. According to several studies[87-90], steroids have a renoprotective effect. In some of these studies, the beneficial effect seems to be related to a long course therapy or to a higher dose. Other studies did not confirm a steroid related beneficial effect[91] or highlight the problem of corticosteroid side effects[92].

A Cochrane review on immunosuppressive therapy in IgAN[93] analyzed 32 studies comprising 1781 patients. Six of these studies analyzed the effects of steroids. A renoprotective effect was observed comparing steroids *vs* placebo or no treatment. Unfortunately, all the aforementioned studies did not answer a number of questions such as the following: Were steroids also effective for patients with a GFR < 30 mL/min per 1.73 m^2? What is the best steroid dosage and regimen to avoid side effects? RCTs that are ongoing such as the Supportive Versus Immunosuppressive Therapy of Progressive IgA Nephropathy (STOP IgAN)[94] and the Therapeutic Evaluation of Steroids in IgA Nephropathy (TESTING) study[95] might provide definitive evidence for a role of corticosteroids in the treatment of IgAN.

Recently, the VALIGA study retrospectively evaluated the role of corticosteroids in IgAN[96]. The authors observed that corticosteroids reduced proteinuria and the rate of renal function decline. In addition, these benefits also involved patients with an eGFR < 50 mL/min. The results of this study should encourage nephrologists to further investigate corticosteroids efficacy in patients with low baseline GFR[97].

Corticosteroids in association with other therapies

The already cited Cochrane review[93] highlighted the higher efficacy of corticosteroids given in association with ARB with respect to corticosteroids alone or ARB alone.

Other studies[80] documented the higher efficacy of tonsillectomy plus steroids with respect to tonsillectomy alone or steroid therapies alone.

The association of steroids with other immunosuppressants has been principally used for high risk patients.

Association of cyclophosphamide and corticosteroids offered different results

The association of cyclophosphamide and corticosteroids has been principally examined in studies concerning patients with progressive renal deterioration or with crescentic IgAN[98-100]. The combined cyclophosphamide/steroid therapy may benefit patients at a high risk of renal failure. The limitation of these studies is that they are small, often retrospective, and side effects represent a serious concern. The KDIGO guidelines[34] do not recommend such treatment for the vast majority of IgAN patients. A possible role is suggested by the guidelines only for patients with crescentic IgAN and rapidly decreasing renal function.

Similarly, the use of azathioprine (AZA) in addition to corticosteroids is not recommended. Indeed, in two studies from Pozzi *et al*[101,102] the addition of AZA to corticosteroids did not provide any beneficial result in patients with ongoing severe chronic renal failure.

The aforementioned Cochrane review on immunosuppressants in the treatment of IgAN highlighted that the use of such treatments had low evidence and was not powerful to guide clinical practice. In addition, evidence on mortality, infections and cancers is sparse or of low quality.

The use of calcineurine inhibitors in addition to corticosteroids has been tested in some recent small RCTs[103,104]. Some benefit has been reported for the reduction of proteinuria, but the addition of cyclosporine in some patients caused a serum creatinine increase and a higher infection incidence.

Other immunosuppressants

In a recent study, Kim *et al*[105] compared tacrolimus (TAC) with ACEI/ARB therapy. In this small study, TAC reduced proteinuria in IgAN patients, but the follow-up was too short to draw any conclusion.

Mycophenolic mofetil (MMF), in addition to its immunosuppressive action on lymphocytes, has been documented to reverse IgA1 aberrant glycosylation, up-regulating the core 1 beta 3 - GalT-specific molecular chaperone that is impaired in IgAN[106].

The first RCT of MMF was conducted on Chinese patients with severe IgAN[107]. The effects on proteinuria were significant at 18 mo. At the same time, two other European studies failed to document a beneficial effect of MMF[108,109]. These data raised the possibility of a different response to MMF in different ancestral cohorts.

Later on, three other Chinese studies reported an improved outcome in IgAN patients treated by MMF[110-112]. In addition to the improved outcomes of patients treated with MMF, the study by Tang *et al*[111] documented that MMF inhibited IgA binding to mesangial cells. Diverging results have also been reported in more recent studies. In an Italian study, MMF and steroids reduced proteinuria and improved outcomes in IgAN patients at risk for progression[113]. In another study, MMF therapy was effective for IgAN children with nephritic syndrome and resistant to steroid treatment[114].

A recent study from the United States was not able to document any MMF related beneficial effect[115], but the study had the limitation of enrolling few patients and had a short follow-up.

A Chinese review on the efficacy and safety of MMF treatment in IgAN recognized that high quality RCTs with large sample sizes and a long follow-up are needed to evaluate the MMF efficacy in IgAN[116]. To date, the KDIGO guidelines do not recommend the use of MMF in IgAN patients.

Therapy for recurrence of IgAN after kidney transplantation

Post-transplant recurrence of IgAN is common. As prevention and treatment of acute and chronic rejection is continuously improving, renal disease recurrence on the graft may become a relevant cause of graft loss over the long term[117]. However, none of the current available immunosuppressive drugs are able to prevent the histological recurrence of IgAN[118,119]. Patients with recurrent IgAN after transplantation should be given optimized supportive care. A Japanese study suggested that a preoperative tonsillectomy might not affect the recurrence of IgAN[120].

A study from Berthoux *et al*[121] suggested that an induction therapy with ATG might have a protective role against IgAN recurrence, but these results have not been confirmed. Registry data from the Australia and New Zealand Dialysis and Transplant Registry documented that the corticosteroids given continuously after transplantation significantly reduced the risk of IgAN recurrence[122]. An analysis of the United States Renal Data System similarly documented a protective effect of corticosteroids after IgAN recurrence in renal transplant patients[123]. A retrospective study documented no benefit using MMF instead of AZA as an antimetabolite drug after transplantation[124].

New therapies and ongoing clinical trials

New therapies: *In vitro* studies documented that peroxisome proliferator-activated receptor-gamma (PPAR-gamma) agonist attenuates inflammation in tubular epithelial cells in IgAN[125]. The additive effect of PPAR-gamma agonist and ARB has been confirmed in an animal model of IgAN[126].

A new enteric formulation of the locally acting glucocorticoid budesonide, designed to release the active compound in the ileo-cecal region, has been used to treat IgAN and was effective in reducing proteinuria and slightly increasing eGFR[127] . The aim of a locally releasing compound is to limit the corticosteroid side effects. Based on these data, a multicenter phase Ⅱb trial (NEFIGAN) is currently ongoing in Europe.

Complement activation is involved in IgAN tissue injury. Rituximab has been successfully used as rescue therapy in IgAN with rapid progression[128] . However, in another study, rituximab, given as a single dose at the beginning of the therapy, failed to reduce proteinuria and to inhibit GFR decline[129].

Bortezomib is a proteasome inhibitor approved for the treatment of multiple myeloma and tested to decrease antibody levels in hyperimmune patients in renal transplantation. The rationale for using Bortezomib in the treatment of IgAN relies on the fact that, in IgAN, a switch from proteasome (PS) to immune PS has been observed, suggesting a hyperactivation of the PS system. In addition, an increased nuclear translocation of the p50 active subunit of NF-κB has been observed in these patients[130,131]. A phase Ⅲ clinical trial is to date ongoing.

Spleen tyrosine kinase (SYK) is an intracellular protein tyrosine kinase involved in cell signaling downstream of the immunoreceptors. Recently, the involvement of the SYK in the inhibition of IgA1 stimulation of human mesangial cells and in the pathogenesis of IgAN has been documented[132]. A RCT with a selective oral SYK inhibitor in patients with IgAN is currently ongoing.

Recently in China the efficacy and safety of Leflunomide given in association with steroids has been evaluated. In a first RCT the efficacy of Leflunomide was evaluated in IgAN patients affected by nephritic syndrome[133]. In this context leflunomide resulted a safe and effective drug for the treatment of IgAN. More recently a larger number of IgAN patients were enrolled in a RCT to receive Valsartan combined with clopidogrel and/or leflunomide for the treatment of progressive IgAN[134]. The treatment with Valsartan combined with clopidogrel and leflunomide resulted in a reduction of proteinuria and of renal function deterioration.

Ongoing clinical trials for IgAN treatment: Several clinical trials for IgAN are ongoing. As mentioned previously, only the active ongoing clinical trials that are recruiting patients will be discussed.

Clinical trials may involve old drugs given with new strategies or new drugs not yet on the market.

Two RCTs are testing the efficacy of MMF in patients with IgAN. One RCT[135] includes patients with proteinuria > 1 g/d already in treatment with ARB. The purpose of the RCT is to evaluate the efficacy of MMF in reducing proteinuria and preserving renal function compared to corticosteroids. The other trial (MAIN)[136] is enrolling patients with advanced IgAN. The purpose of the study is to evaluate MMF compared to losartan alone in patients treated with the maximum tolerated daily dose of losartan.

Four RCTs are evaluating the effects of corticosteroids on IgAN.

Apart from the already cited TESTING study[95], a Chinese RCT[137] is evaluating the efficacy and safety of steroids in IgAN patients with active pathological lesions. The TOPplus-IgAN RCT[138] is evaluating the effects of prednisone plus cyclophosphamide in patients with advanced stage IgAN and is evaluating combination therapy with respect to corticosteroids alone. The first available data from the STOP-IgA[94] reported that appropriate supportive care blunted the effect of immunosuppression in proteinuric IgAN patients.

The adrenocorticotropic hormone (ACTH) has been used in RCTs for the treatment of several diseases, among which is glomerulonephritis. Indeed, ACTH seems to exert a non-specific antiproteinuric effect rather than a specific effect. Bomback *et al*[139] treated several proteinuric patients, among which 5 patients were affected by IgAN with proteinuria resistant to other therapies.

To date, two studies are testing the gel formulation of ACTH in the treatment of IgAN at a high risk of progression[140].

As mentioned previously, rituximab has been used in the treatment of IgAN. To our knowledge, the only RCT on rituximab[141] is not enrolling patients.

CCX168 is an orally administered, specific small molecule inhibitor of the C5a receptor. Trials with CCX168 are ongoing in the treatment of the atypical hemolytic uremic syndrome and antineutrophils cytoplasmic antibodies vasculitis. A phase Ⅱ study is enrolling patients to evaluate CCX168 efficacy in reducing proteinuria in IgAN with persistent proteinuria despite supportive therapy with a maximally tolerated RAS blocker[142].

Blisibimod is a selective antagonist of the B-cell activating factor and is being tested in lupus nephritis. A RCT (BRIGHT-SC) is evaluating blisibimod in a phase Ⅱ/Ⅲ trial in proteinuric patients affected by IgAN[143].

The aforementioned enteric budesonide is being evaluated for the treatment of IgAN in a European multicenter RCT[144].

A pilot study on Velcade (bortezomib)[145] in IgAN has the purpose of investigating the ability of bortezomib to induce complete or partial remission in patients with severe IgAN.

Fostanatimib is a selective inhibitor of SYK that is involved in the pathogenesis of IgAN. A phase Ⅱ RCT is, to date, ongoing with the purpose of determining whether fostanatimib is safe and effective in the treatment of IgA nephropathy[146].

Finally, two Chinese RCTs are evaluating the efficacy of two traditional Chinese medicines; *i.e.,* Abelmoschus Manihot[147] and Tripterygium Wilfordii HOOK[148], in the treatment of IgAN. The former RCT is comparing the study drug with losartan, and the latter with MMF.

CONCLUSION

Patients affected by IgAN may present extremely different clinical aspects at diagnosis. The disease evolution also may differ ranging from a stable course of disease with no evolution to a disease rapidly evolving to ESRD. Accordingly, the therapeutic approaches may vary from only the need for frequent controls to check for disease evolution to careful supportive care for patients with clinical signs, from urinary abnormalities, hypertension and reduced GFR to intensive treatment in patients with rapid evolution.

Because the so called "silent majority" does not have any disease evolution, the major problem is to identify those patients who will have disease evolution in the future. Histological and biological markers have been proposed in an attempt to identify such patients, but, to date, the clinical markers represent the optimal tool for monitoring IgAN patients.

Patients with stable disease with no sign of disease evolution only need to be monitored. Patients with slow evolving disease and low level proteinuria, in addition to being monitored, need optimal supportive care. In recent years, treatment with corticosteroids may be useful for such patients and is recommended by the guidelines.

Intensive treatment with corticosteroids and other immunosuppressants should only be reserved for patients with rapidly progressive disease or with a histological picture of extracapillary glomerulonephritis or with nephrotic proteinuria.

Several RCTs concerning new drugs are included in the international registries, but only some trials are enrolling patients.

In any case, these new drugs should be reserved for high risk patients and should not be used until validated in large studies for a long period of time.

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REVIEW

How do kinases contribute to tonicity-dependent regulation of the transcription factor NFAT5?

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Abstract

NFAT5 plays a critical role in maintaining the renal functions. Its dis-regulation in the kidney leads to or is associated with certain renal diseases or disorders, most notably the urinary concentration defect. Hypertonicity, which the kidney medulla is normally exposed to, activates NFAT5 through phosphorylation of a signaling molecule or NFAT5 itself. Hypotonicity inhibits NFAT5 through a similar mechanism. More than a dozen of protein and lipid kinases have been identified to contribute to tonicity-dependent regulation of NFAT5. Hypertonicity activates NFAT5 by increasing its nuclear localization and transactivating activity in the early phase and protein abundance in the late phase. The known mechanism for inhibition of NFAT5 by hypotonicity is a decrease of nuclear NFAT5. The present article reviews the effect of each kinase on NFAT5 nuclear localization, transactivation and protein abundance, and the relationship among these kinases, if known. Cyclosporine A and tacrolimus suppress immune reactions by inhibiting the phosphatase calcineurin-dependent activation of NFAT1. It is hoped that this review would stimulate the interest to seek explanations from the NFAT5 regulatory pathways for certain clinical presentations and to explore novel therapeutic approaches based on the pathways. On the basic science front, this review raises two interesting questions. The first one is how these kinases can specifically signal to NFAT5 in the context of hypertonicity or hypotonicity, because they also regulate other cellular activities and even opposite activities in some cases. The second one is why these many kinases, some of which might have redundant functions, are needed to regulate NFAT5 activity. This review reiterates the concept of signaling through cooperation. Cells need these kinases working in a coordinated way to provide the signaling specificity that is lacking in the individual one. Redundancy in regulation of NFAT5 is a critical strategy for cells to maintain robustness against hypertonic or hypotonic stress.

Key words: Tonicity enhancer binding protein; Osmotic response element binding protein; Phosphorylation; Kidney; Urinary concentration; Signal transduction; Nephropathy; Hypertonicity; Hypotonicity

Core tip: NFAT5 is critical for kidney functions. Its dis-regulation results in or is associated with the renal diseases and disorders. More than a dozen of kinases have been identified to contribute to tonicity-dependent regulation of NFAT5. The present review is focused on how these kinases regulate NFAT5 activity under the context of hypertonicity or hypotonicity. Understanding these regulatory mechanisms will have therapeutic implications. A precedent example is that recognition of the cyclosporine immunosuppressive effect resulted from inhibition of the phosphatase calcineurin-dependent activation of NFAT1 allows combination use of cyclo-sporine with other mechanistically different immuno-suppressants to improve their therapeutic efficacy and reduce their side effects.

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INTRODUCTION

Functions of NFAT5 in the kidney

The kidney medulla contributes to maintaining body fluid and electrolyte balance through concentration of urine. In order to achieve this goal, the medulla must establish two pre-requisites: Adequate water permeability alone the renal tubules and hypertonicity and hyperosmolality in the renal medullary interstitial fluid, which provide an osmolar gradient driving water absorption. NFAT5, nuclear factor of activated T cells 5[1] also named as TonEBP[2] and OREBP[3], is the primary transcription factor that is activated by hypertonicity in the mammalian system and plays a pivotal role in establishing these two conditions. NFAT5 activates expression of water channels aquaporin-2 (AQP-2), which dictates the apical water permeability of the collecting ducts[4-6] and aquaporin-1 (AQP-1), an important gene for water trafficking across the proximal tubules and descending limb of the loop of Henle[7], and urea transporter 1 (UTA1), a critical contributor for hyperosmolality in the renal medullary interstitium[5,6,8], and osmoprotective genes like betaine/glycine transporter 1 (BGT1), sodium-dependent myo-inositol transporter (SMIT) and aldose reductase (AR)[1-3,9], which are essential for the kidney medulla to survive in the hypertonic environment. Expression of a dominant negative mutant of NFAT5 in the kidney epithelial cells reduces expression of AQP-2 and UTA1 and impairs urinary concentration[5]. A majority of homozygous NFAT5 knockouts die embryonically[5], probably due to impaired development and function of cardiomycytes[10]. The survived knockouts have profound renal medullary hypotrophy with reduced expression of the osmoprotective gene[9]. Thus, NFAT5 is tightly regulated in the kidney medulla to ensure normal process of urinary concentration. Hypokalemia, cyclosporine A and lipopolysaccharides-induced urinary concentration defect is associated with reduced NFAT5 activity in the region[6,11,12]. Water restriction induces an increase of urinary excretion of sodium to prevent hypernatremia and rise in extracellular tonicity. In the primary rat renal medullary cells, NFAT5 is necessary for hypertonicity-induced increase of serum- and glucocorticoid-inducible kinase-dependent expression of the type A natriuretic peptide receptor[13]. This cascade might be a mechanism for dehydration-induced natriuresis[13].

Besides activation by hypertonicity, NFAT5 is also activated by hypoxia[14,15]. Renal ischemia for 30 min increases the mouse medullary mRNA abundance of NFAT5, which is protective against ischemia/reperfusion-induced acute kidney injury[14]. However, ischemia for 45 min in the rat kidney decreases NFAT5 mRNA and protein abundance in the medulla[16], but the functional consequence of the effect remains unknown[16]. NFAT5 mRNA is up-regulated in the kidney by unilateral ureteral obstruction[17]. NFAT5 involves in diabetic nephropathy. Haplotype association analysis of 718 type 1 diabetic patients reveals a significant association of NFAT5 with nephropathy[18]. High glucose increases NFAT5 transcriptional activity more in the peripheral blood mononuclear cells isolated from type 1 diabetes patients with nephropathy than in the cells isolated from the patients without nephropathy[19].

Phosphorylation of NFAT5

NFAT5 belongs to the family of the Rel transcription factors, including NFAT1-4 and NF-κB[1-3]. It is best known for its essential role in protecting cells from hypertonic stress. However, it has become clear that NFAT5 also has important functions outside hyperto-nicity[20]. Therefore, it is not surprising that NFAT5 is also expressed in the tissues that are not normally exposed to hypertonicity[21]. Hypertonicity activates NFAT5 by increasing its transactivation, nuclear localization and DNA binding and protein abundance[22]. Like many other biological processes, phosphorylation of NFAT5 regulates NFAT5 activation. High NaCl rapidly increases phosphorylation of NFAT5. NFAT5 has 216 serines, 15 tyrosines, and 111 threonines, all of which could be phosphorylated[22]. Through mass spectrometry, DNA mutation, immunocytochemistry and Western analyses, NFAT5 tyrosine 143 (Y143), threonine 135 (T135), serine 155 and 158 (S155 and S158) have been identified so far as the phosphorylation sites and play a critical role in regulation of NFAT5 activity. High NaCl increases phosphorylation of NFAT5-Y143, leading to increase of NFAT5 nuclear localization in cell culture[23-25], and phosphorylation of NFAT5-Y143 is increased in the normal rat renal inner medulla and the Brattleboro rat inner medulla treated with vasopressin, known to increase the renal medullary tonicity[25]. The similar

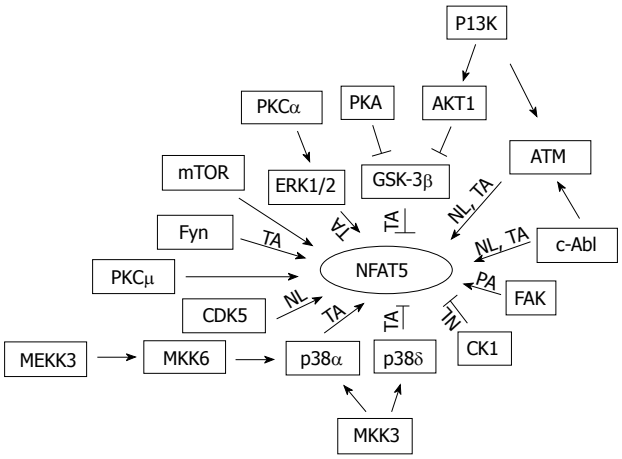

Figure 1 Summary of kinases known to regulate tonicity-dependent activation/inactivation of NFAT5 through an increase/decrease of its transactivating activity, nuclear localization and/or protein abundance. If none of these three steps appears with an arrow, this means that the mechanism is unknown. mTOR: Mammalian target of rapamycin; TA: Transactivating activity; NL: Nuclear localization; PA: Protein abundance; PKA: Protein kinase A; PKC: Protein kinase C; ERK: Extracellular signal-regulated kinase; CDK: Cyclin dependent kinase.

phenomena are also observed with NFAT5-T135[26]. On the other hand, low NaCl increases phosphorylation of NFAT5-S155 and then S158, leading to reduced NFAT5 nuclear accumulation[26,27]. In contrast to the demonstration of regulation of NFAT5 nuclear distribution by direct phosphorylation, how phosphorylation regulates NFAT5 transactivating activity is elusive. Although a majority of kinases contributes to tonicity-dependent increase of NFAT5 transactivation (Figure 1), none of phosphorylation sites in the transactivation domain has been definitively identified. NFAT5-S1197, S1247 and S1367 lie in the NFAT5 transactivation domain. Over expression of the alanine mutants of these serine residues in HEK293 cells or AT cells, which have inactive ATM kinase, reduces NFAT5 transcriptional activity under isotonicity and/or hypertonicity[28]. However, whether high NaCl increases phosphorylation of these serine residues remains unknown.

Signaling regulation through coordination

Consistent with the observations that phosphorylation regulates NFAT5 activation are that more than a dozen of kinases (Figure 1) and a few phosphatases have been identified to regulate NFAT5 transcriptional activity[22,23,29-32]. However, all of these kinases and phosphatases also regulate other cellular activities and even opposite activities. For example, p38 and ERK1/2 contribute to hypertonicity-induced activation of NFAT5, but hypotonicity, which is known to inhibit NFAT5 activity, also increases phosphorylation (activation) of these two kinases[30]. The pleiotropic effects of these kinases and phosphatases raise a question concerning how they can selectively signal to NFAT5 in the context of hypertonicity. Another question is why many signaling molecules are needed to regulate NFAT5 activity. Signaling through cooperation/committee might be a plausible explanation. This concept was originally put forward to describe how protein kinases and phosphatases in budding yeast capture and relay information in a coordinated way responding to a signal[33]. This concept can be viewed as that cells have a specific committee tasked for a specific perturbation. Each member in the committee is predecided when, where and how to act, so that cells can respond to the perturbation in a coordinated way[34]. The committee members are like different and redundant instrument players in an orchestra in which each one plays his/her instrument, maybe viewed as activation of a signaling molecule, in a coordinated way with other players for a specific music piece signaled by a conductor. This theory explains why each of the identified kinases is necessary for full activation of NFAT5, but none alone, is sufficient[22], why CDK5 is only required in the early phase of NFAT5 activation[26], and why over expression of catalytically active PKA only increases NFAT5 activity under isotonicity but not under hypertonicity[35], because neither a single player nor an over active player can play an orchestra piece. Numerous signaling molecules are required in order to form redundancy in signaling hypertonic stress. Redundancy is a critical strategy for cells to maintain robustness against internal and external perturbations[36], as multiple players are needed to produce desired volume from a particular instrument in orchestrating a music piece.

Potential clinical significance of this review

The present review is focused on how kinases contribute to tonicity-dependent activation/inactivation of NFAT5, since this area is the most studied one. Understanding these regulatory pathways will have therapeutic implications. A good example is the mechanism by which cyclosporine A and tacrolimus suppress immune reactions. These two medications inhibit the phosphatase calcineurin, which leads to inhibition of nuclear translocation of NFAT1, NFAT2 and NFAT4, resulting in suppression of expression of the proinflamatory cytokines[37]. This mechanism has helped understanding both the therapeutic and side effects including renal toxic effects of the medications[37] and is critical for the combined use of cyclosporine A and tacrolimus with other mechanistically different immunosuppressants to improve their therapeutic efficacy and reduce their side effects[38]. Interestingly, cyclosporine A induces urinary concentration defect, which is ascribed to the decrease in NFAT5 activity in the rat kidney[12,39]. The effect is mediated through inhibition of calcineurin remains unknown. Another example is that the anti-diabetes medication metformin was shown to induce apoptosis in the kidney medulla of both normally hydrated and dehydrated type 2 diabetic mice, probably by inhibition of NFAT5 through activation of 5'-AMP-activated protein kinase[40]. This observation raises safety concern for metformin in the dehydrated diabetic patients[41].

Diabetic nephropathy is one of the most severe

complications of diabetes with resultant increases of morbidity and mortality. Its treatment has posed a formidable challenge to the medical and scientific communities. Numerous novel therapeutic approaches promisingly found in animal studies have not been successfully translated into clinical practices[42]. For example, pyridoxamine, which showed a potent effect in blocking formation of advanced glycosylated end product in animal models has failed in clinical trials[42,43]. AR, a transcriptional target of NFAT5, is a rate-limiting enzyme of the polyol pathway, which plays a crucial role in the pathogenesis of diabetic complications including diabetic nephropathy[44,45]. Thus, targeting the regulatory network of NFAT5 may be an alternative approach to treat diabetic nephropathy. In this regard, the extract from plant Aralia elata has been recently shown to prevent neuronal death by downregulating NFAT5 and AR in mice with diabetic retinopathy, although which regulatory pathway it affects remains unknown[46].

Mitogen-activated protein kinases

Mitogen-activated protein kinases (MAPKs) have three major families: p38, extracellular signal-regulated kinases (ERKs) and c-Jun NH_2-terminal protein kinases (JNKs). Each family has multiple isoforms. They are the most studied kinases in tonicity-dependent activation of NFAT5, which was recently reviewed[30]. The present review only summarizes the salient points of that review. p38 has four isoforms: p38α[47], p38β[48], p38γ[49] and p38δ[50]. p38α contributes to tonicity-dependent activation of NFAT5, whereas p38δ does the opposite[51]. The imidazole derivatives such as SB203580 inhibit p38α and p38β, but not p38δ[50,52]. SB203580, its analogs, p38α dominant negative mutant or siRNAs uniformly inhibits hypertonicity-induced NFAT5 transcriptional activity[53-63]. Because p38α is also called p38, it has been often concluded that p38 signals hypertonicity-induced activation of NFAT5. However, this conclusion causes confusion for interpretation of the effect of the p38 upstream kinase MKK3 and phosphatase MKP-1 on NFAT5 activity. Over expression of MKK3 dominant negative mutant[64] or MKP-1[51] inhibits p38 without significantly affecting NFAT5 transcriptional activity. This paradox can be explained by the interpretation that inhibition of the positive effect of p38α by SB203580, a dominant negative mutant[54] or its siRNAs[51] unmasks an inhibitory effect of p38δ, whereas the dominant negative mutant of MKK3[50,65] and MKP-1[51] reduce both p38α and p38δ activities, therefore, causing no significant change in NFAT5 activity[51,64]. Based on this theory, it is not surprising that another p38 upstream kinase MKK6[13,66] and the MKK3 and MKK6 upstream kinase MEKK3[67] have been demonstrated to contribute to tonicity-dependent activation of NFAT5, because although both MKK3 and MKK6 activate p38α under hypertonicity[68,69], MKK3 strongly activates p38δ, whereas MKK6 does not[70].

Although p38 is the most studied MAPKs in the context of tonicity-dependent regulation of NFAT5, the exact mechanism underlying this effect is far from clear. Whether p38 is critical for tonicity-dependent activation of the transcription factor is even questionable. Knockdown of Rac1 or OSM by its siRNAs reduces high NaCl-induced NFAT5 transcriptional activity, but increases phosphorylation of p38 at both basal and hypertonic levels in HEK293 cells[66]. It should be noted that an opposite effect of knockdown of Rac1 or OSM on phosphorylation of p38 in the same type of cells was reported[68]. Although whether activation of p38 is regulated by cell volume or intracellular ionic strength remains unclear, hypotonicity, which reduces nuclear NFAT5, presumably NFAT5 activity[27,71], also activates p38 in various types of cells[72-75]. These observations call for more attention to which isoform of p38 when the effect of the kinase on NFAT5 is examined.

The chemical inhibitors of MEK-ERK1/2 PD98059 and U-0126 inhibit high NaCl-induced activation of NFAT5 in nucleus pulposus cells[62], renal carcinoma cells[55] and possibly in mIMCD3 cells[76]. ERK2 siRNA reduces high NaCl-dependent NFAT5 transcriptional activity in nucleus pulposus[62] and in HEK293 cells[29]. It is reasonably concluded that ERK1/2, or at least ERK2, contributes to tonicity-dependent activation of NFAT5[30], although it is not clear why PD98059 fails to inhibit NFAT5 transcriptional activity in the primary splenocytes[61]. The effect of JNK on tonicity-dependent activation of NFAT5 is elusive and also least studied. Both lack of effect[64] and a positive effect of JNK1/2[55] on NFAT5 have been reported.

Like the effect on p38, hypotonicity also increases phosphorylation of ERK1/2 in human keratinocytes[73], mIMCD3 cells[77], renal epithelial A6 cells[72], although inhibition of ERK by hypotonic stress in A6 cells was also reported[78]. Therefore, the mechanism for how ERK1/2 contributes to tonicity-dependent activation of NFAT5 remains to be elucidated. In an overly simplified term, p38 and ERK1/2 can signal both hypertonic and hypotonic responses, depending on which committee they are in.

AGC protein kinases

Based on sequence alignments of the catalytic domains, the term AGC kinase was first used in 1995 to define a subgroup of serine/threonine protein kinases that were most related to cAMP-dependent protein kinase 1 (PKA; also known as PKAC), cGMP-dependent protein kinase (PKG; also known as CGK1α) and protein kinase C (PKC)[79]. It was later realized that the group of AGC protein kinases includes more than 60 protein kinases in the human genome, classified into 14 families: PDK1, AKT/PKB, SGK, PKA, PKG, PKC, PKN/PRK, RSK, NDR, MAST, YANK, DMPK, GRK and SGK494[80]. AGC kinases regulate a wide array of important cellular functions. Therefore, their mutation and dysregulation contribute to the pathogenesis of various human diseases, including kidney diseases[80].

(1) PKA exists as a heterotetramer composed of two regulatory subunits and two catalytic subunits. A pseudosubstrate motif in the regular subunits binds to the substrate-binding site of the catalytic domain. Upon activation, two molecules of cAMP bind to each regulatory subunit, allowing the release of active catalytic subunits[80]. PKA is the first AGC kinase demonstrated contributing to tonicity-dependent activation of NFAT5[35]. Hypertonicity induced by high NaCl increases PKA activity. An inhibitor of PKA (H89, 10 μmol/L) and dominant-negative PKA catalytic subunit reduce NFAT5 transcriptional activity associated with a decrease of NFAT5 transactivating activity in HepG2 cells[35]. Further, overexpression of the catalytic subunit of PKA (PKAc) alone increases NFAT5 transactivating and transcriptional activities under the isotonic condition[35]. Subsequent studies indicate that PKA contributes to tonicity-dependent activation of NFAT5 by suppressing the negative effect of GSK-3β on the transcription factor through increasing the inhibitory phosphorylation of GSK-3β at serine 9[81]. PKA has also been suggested to contribute to tonicity-dependent activation of NFAT5 in the primary splenocytes, based on the inhibitory effect of H89[61]. H89 at 2 μmol/L failed to inhibit high NaCl-induced increase of protein abundance of HSP70, a transcriptional target of NFAT5[82,83], in NIH3T3 cells[84]. This is probably due to that the concentration of H89 is too low. Whether hypertonicity-induced activation of PKA requires cAMP is not clear. High NaCl does not significantly alter cAMP level in HepG2 cells where the effect of PKA on NFAT5 is observed[35] or in LLC-PK1 cells[85], but increases cAMP level in mIMCD3 cells[86] and neutrophils[87]. Yet, the lack of the effect of forskolin (increasing intracellular cAMP) or dibutyryl-cAMP (a mimic of cAMP) on NFAT5 transcriptional activity in HepG2 cells let investigators conclude that the effect of PKA on NFAT5 is cAMP-independent[35]. A precedent example is that activation of NF-κB by PKA is independent of cAMP[35]. It is not clear why hypertonicity does not increase PKA activity in mpkCCD14 cells[88].

(2) The PKC family has 10 isoforms and can be divided into three categories based on their structure and biochemical properties: classical or conventional PKC (cPKC), including PKCα, PKCβ Ⅰ, PKCβ Ⅱ, and PKCγ; novel PKC (nPKC), including PKCδ, PKCε, PKCη, and PKCθ; and atypical PKC (aPKC), including PKCζ and PKCλ. PKC is a primary target of diacylglycerol, which is produced by phospholipase C (PLC)-catalyzed hydrolysis of lipid phosphatidylinositol-(4,5)-bisphosphate [Ptd-Ins(4,5)P2]. Diacylglycerol binds a conserved C1 domain in PKC, resulting in the plasma membrane translocation and activation of the kinase[89]. High NaCl increases PLCγ1 activity[24], diacylglycerol and total PKC activity[90]. PKC inhibitors reduce NFAT5 transcriptional activity in mIMCD3[91] and NIH3T3 cells[84]. We recently identified PKCα involved in regulation of NFAT5 activity[29]. Acute hypertonic stress with high NaCl increases PKCα activity in HEK293 cells. Knockdown of PKCα by its siRNAs decreases NFAT5 transcriptional activity mediated by reduction of NFAT5 transactivating activity, but not by NFAT5 nuclear localization or protein abundance[29]. More interestingly, PKCα activity is elevated in the kidney inner medulla due to increase of its protein abundance. Knockout of PKCα reduces expression of NFAT5-targeted genes AR and betain/glycine transporter 1, associated with reduced expression of NFAT5 protein abundance[29]. This is the first demonstration showing that a signaling molecule regulates NFAT5 in the kidney inner medulla. The effect of PKCα on NFAT5 is relayed by ERK1/2 in HEK293 cells and possible in the kidney inner medulla, since knockdown of PKCα attenuates high NaCl-induced phosphorylation of ERK1/2 and has no additional inhibition on NFAT5 in the presence of ERK2 siRNAs in HEK293 cells, and knockout of the kinase reduces phosphorylation of ERK1/2 in the kidney inner medulla[29]. PKCα was previously demonstrated to contribute to regulation of urinary concentration[92,93], possibly by increasing high NaCl-dependent phosphorylation of urea transporters[93] and urea permeability[94] in the inner medullary collecting ducts. Our recent observations provide a possible additional mechanism for the effect of PKC on urinary concentration[29].

PKD1, also called PKCmu, is one of three members of PKD kinase family that is closely related to PKC. PKC activates PKD through direct phosphorylation of S744 and S748 in the activation loop of PKD[95]. PKD is highly mobile and functions as a "communicator"between different subcellular compartments[95]. General PKC inhibitors, Go6976 and GF109203X, and siRNA-mediated knockdown of PKD1 reduce high NaCl-induced increase of protein abundance of HSP70[84]. The general inhibitors reduce high NaCl-induced NFAT5 mobility shift and have no significant effect on NFAT5 nuclear localization[84]. The latter effect is consistent with the lack of effect of PKCα and ERK1/2 on NFAT5 nuclear accumulation. PKD acts upstream of ERK1/2 under certain contexts[95]. However, it remains unclear whether PKD1 involves in PKCα-ERK1/2 signaling activation of NFAT5, since elimination of high NaCl-induced phosphorylation of ERK1/2 by PD98059 (20 micromol/L) does not reduce tonicity-dependent increase of HSP70 protein abundance[84].

(3) The AKT protein kinase family comprises three highly related isoforms encoded by different genes. Despite the shared common, multi-step mechanism of activation downstream of class IA PI3 kinases, these isoforms play different roles in signaling, as revealed by distinct phenotypes displayed by genetically modified animals, identification of isoform-specific substrates and association with discrete subcellular locations[96]. Inhibition of phosphorylation of AKT1-S473 by a general AKT inhibitor, triciribine, or by a PI3K inhibitor wortmannin reduces high NaCl-induced expression of AR, BGT1 and SMIT[97]. Co-expression of the catalytically active AKT1 with GSK3 in the GSK3$^{-/-}$ mouse embryonic fibroblasts reverses the inhibitory effect of GSK3β on NFAT5. These data indicate that AKT1 contributes to tonicity-dependent activation of NFAT5 by attenuating the inhibitory effect of GSK3[81]. Whether

hypertonicity/hyperosmolality activates AKT remains controversial. Hypertonicity activates AKT, including direct measurements of increased AKT activity in NIH 3T3 and CHO cells[98]. High NaCl increases phosphorylation (activation) of AKT1-S473 in mCCD$_{cl1}$ and HepG2 cells[97]. Also, high NaCl increases phosphorylation of AKT-S174 in Madin-Darby canine kidney cells, so does dehydration in the rat inner medulla[99]. In contrast, high sorbitol decreases the kinase activity in HEK293 and COS cells[100], and high sucrose decreases the kinase activity in Swiss 3T3 cells, despite increases of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) abundance and PI3K activity[101]. In the latter study, failure of the increased PIP3 to activate AKT was ascribed to concomitant activation of an inhibitory pathway. It is worthwhile to note that these studies were done with different hypertonicity/hyperosmolality-inducers in different types of cells.

Ataxia telangiectasia-mutated, c-Abl and phosphatidylinositide 3-kinase-IA

Ataxia telangiectasia-mutated (ATM) is a DNA damage-inducible serine/threonine kinase belonging to the PI3K-like kinase family[102]. PI3K is a family of lipid kinases that phosphorylate the 3'-position hydroxyl of the D-myo-inositol head group to generate specific phosphoinositide forms[103]. Based on their *in vitro* lipid substrate specificity, structure, and mode of regulation, PI3Ks can be divided into three main classes. Class Ⅰ, which has class Ⅰ A and B, synthesizes phosphatidylinositol (3,4)-bisphosphate [PtdIns(3,4)P2] and phosphatidylinositol (3,4,5)-trisphosphate [PtdIns (3,4,5) P3][103]. It is a heterodimer composed of a p110 catalytic subunit and a p85 regulatory subunit[103]. c-Abl belongs to a family of non-receptor tyrosine kinases, which has two members, c-Abl and Arg (Abl-related gene)[104]. These three different types of kinases are reviewed together, because evidence already exists that they act in coordination to regulate high NaCl-induced activation of NFAT5. It has been proposed for a while that hypertonicity/hyperosmolality-induced damages interplay with hypertonicity/hyperosmolality-induced responses[22,105,106]. The role of ATM in regulation of NFAT5 activity is an example of this theory. High NaCl damages DNA[107]. High NaCl activates ATM, most likely through high NaCl-induced DNA damage, although it is difficult to directly approve it[28]. ATM contributes to high NaCl-induced activation of NFAT5 through increasing NFAT5 transactivating activity[28] and nuclear localization[108]. Phosphatidylinositide 3-kinase-IA (PI3K-IA) contributes to tonicity-dependent activation of NFAT5 by increasing its transactivation, since over expression of a dominant negative mutant of p85 or by siRNA-mediated knockdown of p110α reduces NFAT5 transcriptional and transactivating activities[109]. PI3K-IA acts as an upstream kinase to mediate high NaCl- and ionizing radiation-induced activation of ATM as measured by the stimulatory phosphorylation of ATM[109]. Since NaCl-induced increase of NFAT5 activity is reduced equally by inhibition of ATM and PI3K-IA, and the effects are not additive, it is concluded that the effect of PI3K-IA on tonicity-dependent activation of NFAT5 is mediated by ATM[109]. However, it is not clear why PI3K-IA is not involved in high NaCl-induced increase of nuclear NFAT5[109]. High NaCl increases c-Abl kinase activity. Like ATM, c-Abl regulates tonicity-dependent activation of NFAT5 through increasing NFAT5 transactivating activity and nuclear localization[25]. The effect of c-Abl on NFAT5 nuclear distribution is also mediated by direct phosphorylation of NFAT5-Y143[25]. Over expression of a c-Abl kinase dead mutant abolishes high NaCl-induced phosphorylation (activation) of S1981 of ATM, and high NaCl-induced NFAT5 nuclear accumulation is greatly enhanced in AT cells, which lack active ATM, when wild-type ATM is transfected[25]. These data indicate that c-Abl regulates NFAT5 activity through ATM. However, it is unlikely that the protein tyrosine kinase c-Abl directly phosphorylates ATM-S1981. Further, the relationship between PI3K-IA and c-Abl in signaling activation of NFAT5 remains unknown.

Mammalian target of rapamycin

Mammalian target of rapamycin (mTOR) is a serine-threonine kinase belonging to the phosphatidylinositol kinase-related kinase family[110]. It has two multi-protein complex isoforms, mTORC1 and mTORC2. The mTORC1 is composed of regulatory-associated protein of mTOR (Raptor), PRAS40 (also known as Akt substrate 1) and mLST8. mTORC1 is rapamycin-sensitive. mTORC2 combines rapamycin-insensitive companion of mTOR, mSIN1, Protor and mLST8[110]. mTOR controls cell growth and division in part through regulating ribosomal p70 S6 kinase and the eukaryotic translation initiation factor 4E binding proteins[110]. It is well-known that mTORC1 is activated by PI3K-AKT axis[110]. Whether this mechanism is also present in the hypertonic setting is not clear. High NaCl increases PI3K-IA kinase activity in HEK293 cells[109] and phosphorylation (activation) of AKT1-S473 in mCCD$_{cl1}$ or HepG2 cells[97], but analyses of diagnostic substrates downstream mTORC1 by phosphorylated-S235/236 in the ribosomal subunit S6, and phosphorylation-dependent electrophoretic mobility shift of 4E-BP1 and mTORC2 by phosphorylation of S473 of AKT show that hypertonicity partially inhibits both complexes in the immortalized wild-type adenosine monophosphate-activated protein kinase (AMPK) mouse embryonic fibroblasts[111]. The discrepancy could be due to different types of cells used. Nevertheless, based on the inhibitory effects of the mTOR inhibitors, torin1 and rapamycin, on high NaCl-induced expression of NFAT5-targeted genes and NFAT5 transcriptional reporter activity, it is concluded that mTOR contributes to tonicity-dependent activation of NFAT5. The effect of mTOR is probably due to facilitating a transcription-permissive condition for NFAT5 by enhancing histone H4 acetylation and the recruitment of RNA polymerase Ⅱ[111]. It should be pointed out that in human colon cancer cell lines under an isotonic condition, NFAT5 activates expression of a DNA damage-response kinase, REDD1, which in

turn inhibits mTOR signaling[112].

Src family kinases

Src kinase is a family of non-receptor tyrosine kinases that regulate a wide variety of cellular activities such as cell adhesion and motility, carcinogenesis, immune cell function, and even learning and memory. This family has 12 members: c-Src, Fyn, Yes, Yrk, Lyn, Hck, Fgr, Blk, Lck, Brk, Srm, and Frk (with Frk/Rak and Iyk/Bsk subfamilies), 11 of which are found in humans[113]. Src family kinases exhibit a common modular architecture dominated by so-called "SRC homology," or SH domain. SH1 is the catalytic domain. In the inactive state, a key tyrosine in this domain (Y416) blocks the substrate binding site. When autophosphorylated, this residue is displaced and substrate access is unimpeded. SH2 and SH3 are protein-protein interaction domains shared not only among the members but also with many other signaling proteins[114]. The effects of hypertonicity on activities of Src kinases are heterogeneous. Hypertonicity increases Fyn activity and phosphorylation of its targets[115,116], whereas it inhibits c-Src activity[116]. The involvement of Src family kinases in regulation of NFAT5 was suggested by the observation that a Src family kinase inhibitor PP2 reduces NFAT5 transactivating activity and protein abundance in the colon cancer cells[117]. More convincing evidence comes from studies of Fyn. Using PP2, Fyn dominant negative mutant and Fyn null cells, Ko *et al*[54] demonstrated that Fyn contributes to hypertonicity-induced activation of NFAT5 by increasing its transactivating activity.

Focal adhesion kinase

Focal adhesion kinase (FAK) is a mechanosensitive non-receptor protein tyrosine kinase that is widely expressed. In response to integrin engagement as occurs in hypertonicity-induced cell shrinkage, FAK is autophosphorylated and activated at Y397, which entails diverse intracellular events. This function makes FAK a central signaling component downstream of integrin[118]. FAK is abundant in the renal papilla, and furosemide, known to reduce the renal medullary interstitial tonicity, decreases phosphorylation of FAK-Y397 in the region[32]. Hypertonicity increases time-dependent phosphorylation of FAK-Y397 in HEK293 cells[32]. FAK contributes to hypertonicity-induced increase of NFAT5 transcriptional activity[32]. The mechanism underlying this effect is unique, because FAK affects neither hypertonicity-induced increase of nuclear NFAT5 nor NFAT5 transactivating activity. Instead, the effect is mediated by contribution of FAK to hypertonicity-induced increase of NFAT5 protein abundance through stabilizing its mRNA, which depends on NFAT5 3′-UTR[32]. Integrin alpha1beta1 is necessary for hypertonicity-induced full activation of NFAT5 in the inner medullary collecting duct cells[119]. Integrin alpha1-null mice have impaired ability to accumulate organic osmolytes in the inner medulla due to decreased expression of NFAT5-targeted osmoprotective genes and develop early tubular necrosis and increased apoptosis of renal medullary cells following dehydration[119]. Although integrin regulates NFAT5 activity in renal cells and possible in the renal medulla[119] and carcinoma cells[32,117,120,121], whether the effect is through FAK remains to be determined. Besides autophosphorylation at Y397, FAK can be also phosphorylated at multiple tyrosine residues by Src family kinases[118]. FAK is constitutively active in a renal cell carcinoma cell line Caki-1 under an isotonic condition. This is probably due to a high Src kinase activity in the cells[55]. The high activities of Src and FAK in Caki-1 cells are in part responsible for the high basal activity of NFAT5 in the cells as compared with that in the non-cancerous proximal tubule cell line HK-2[55].

Cyclin dependent kinases

The human kinome reveals that the serine/threonine kinase Cyclin dependent kinase (CDK) family has 26 members, of which 21 are classified as CDKs and five form a more distant group of CDK-like kinases[122,123]. CDKs regulate the cell division cycle, apoptosis, transcription and differentiation. Each CDK serves its function by recognizing its specific substrate or other protein effector through the divergent spots located in an overall conserved architecture[122,123]. In HEK293 cells, high NaCl activates CDK5, which directly phosphorylates NFAT5-T135. Phosphorylation of NFAT5-T135 is also increased in the rat renal inner medulla[26]. Inhibition of CDK5 by its siRNA or an inhibitor reduces the increase in NFAT5 transcriptional activity that has occurred by 4 h after NaCl is raised, associated with inhibition of NFAT5 nuclear accumulation at that time, but does not reduce either NFAT5 activity or nuclear NFAT5 after 16 h. This is because high NaCl increases the overall abundance of NFAT5 protein at the later time, which eventually raises its effective level in the nucleus, but the early effect of high NaCl on NFAT5 nuclear localization requires CDK5[26]. CDK5 has no significant effect on NFAT5 transactivating activity. This is special, because a majority of signaling molecules identified so far affects NFAT5 transactivation activity without altering NFAT5 nuclear localization (reviewed above). Besides CDK5, CDK9 also regulates NFAT5. The targeted proteomics shows that CDK9 is physically associated with DDX5/17, a RNA helicase important in alternative RNA splicing of NFAT5. CDK9 is necessary for DDX5 recruitment to NFAT5 as measured by chromatin immunoprecipitation[124].

Glycogen synthase kinase 3β, Casein kinase 1 and 5' -AMPK

In contrast to kinases reviewed above that contribute to high NaCl-induced activation of NFAT5, Glycogen synthase kinase 3β (GSK3β), Casein kinase 1 (CK1) and AMPK actually inhibit tonicity-dependent activation of NFAT5. Therefore, these three kinases are reviewed together. GSK3β is a ubiquitously expressed serine/thre-

onine kinase originally characterized as phosphorylating and inactivating glycogen synthase, the rate-limiting enzyme of glycogen synthesis[125]. Since then, GSK3β has been found to regulate a wide variety of biological processes such as function of neurons[126], immunological responses[127], cardiac hypertrophy[128] and cancer[129]. The pleiotropic effects of GSK3β involve regulation of many transcription factors, such as cAMP response element-binding protein, neurogenin 2, SMAD1, c-Jun, β-catenin[126] and NFAT1-4[127]. GSK3β is unique because unlike most other protein kinases it is most active in cells' resting state, contributing to inhibition of its target transcription factors. When the cells are stimulated, GSK3β is inhibited, resulting in activation of its substrates. The activity of GSK3β is inhibited by phosphorylation of serine residues, of which, serine 9 is most studied[126]. This mechanism is not exceptional in tonicity-dependent activation of NFAT5. GSK3β inhibits NFAT5 transcriptional activity by reducing NFAT5 transactivating activity and protein abundance under the normal tonicity. High NaCl increases phosphorylation of GSK3β-S9 and decreases GSK3β activity, which results in an increase of NFAT5 transcriptional activity mediated by the increment of NFAT5 transactivating activity, but not by NFAT5 nuclear localization or protein abundance[81]. The lack of the effect of GSK3β on NFAT5 nucleo-cytoplasmic trafficking is in contrast to its effect on NFAT1-4. GSK3β phosphorylates the serines in serine-proline repeats, conserved in the amino terminus of NFAT1-4, resulting in promotion of nuclear exit of NFAT1-4 and inhibition of NFAT1-4 transcriptional activity[130]. Unlike NFAT1-4, NFAT5 does not contain serine-proline repeats in its amino terminus[2,131]. Instead, its nucleo-cytoplasmic distribution is regulated by phosphorylation of other amino acids in the terminus such as tyrosine 143[23-25], threonine 135[26] and serines 155 and 158[27]. The difference in amino acid composition explains why GSK-3β affects nuclear localization of NFAT5 differently from that of NFAT1-4.

The stimulatory effect of PKA, PI3K and AKT1 on NFAT5 is dependent on their attenuation of the GSK3β inhibitory effect on the transcription factor[81]. Therefore, GSK3β integrates, at least in part, the effects of PKA, PI3K and AKT1 on NFAT5. However, GSK3β is not involved in the effect of p38α on NFAT5, because co-expression of p38α and its constitutively active upstream kinase MKK6 does not increase phosphorylation of GSK3β-S9 or reverse the inhibitory effect of GSK3β-S9 on NFAT5[81], despite the observations in other settings that p38α inhibits GSK3β activity[132]. On the other hand, low NaCl reduces the inhibitory phosphorylation of GSK3β-S9, which leads to reduction of NFAT5 mRNA and protein abundance in the mouse inner medullary collecting duct cells[133]. It is worth noting that the inhibitory effect of high NaCl on GSK3β may be cell-dependent, because high NaCl reduces the phosphorylation of GSK3β-S9 and increases the kinase activity in several tumor cell lines[134] and decreases the phosphorylation of GSK3β-S9 in the renal medullary interstitial cells[135]. It would be interesting to know the effect of high NaCl on NFAT5 activity in these cells.

CK is a group of serine/threonine kinases that can be divided into CK1 and CK2 families based on their high homology in their catalytic domains[136]. In vertebrates, seven CK1 isoforms (α, β, γ1, γ2, γ3, δ and ε) and several splice variants for CK1α, δ, ε and γ3 have been identified[136]. This family of kinases has been shown to phosphorylate key regulatory molecules involved in a wide array of cellular activities such as cell cycle, cytokinesis, chromosome and microtubule dynamics and transcription and translation[136]. NFAT5 nucleocytoplasmic trafficking is regulated by the dual phosphorylation of serine 155 and 158[27]. Hypotonicity increases phosphorylation of NFAT5-S155, which primes the phosphorylation of serine 158, leading to reduction of nuclear NFAT5[27]. Unlike GSK3β, which has no significant effect on NFAT5 cellular trafficking[81], CK1α1L increases phosphorylation of NFAT5-S158, contributing to hypotonicity-induced decrease of nuclear NFAT5[27].

The serine/threonine kinase AMPK is a major cellular energy sensor that exists as a heterotrimer composed of a catalytic α subunit and each of regulatory β and γ subunits[137]. A high level of AMP or a low level of ATP activates AMPK through phosphorylation of the kinase, resulting in inhibition of energy consumption and stimulation of energy production, which leads to restoration of energy homeostasis[137]. Hypertonicity inhibits the kinase as measured by phosphorylation of the enzyme in the renal medullary interstitial cells (RMIC)[138]. Pharmacological activators of AMPK reduce high NaCl-induced NFAT5 nuclear localization and expression of NFAT5-targeted genes in the cultured RMIC and increases dehydration-induced apoptosis in the mice medulla, suggesting that AMPK inhibits tonicity-dependent activation of NFAT5[138]. Further, the anti-diabetes medication metformin activates AMPK and inhibits NFAT5 transcriptional activity in RMIC and increases RMIC apoptosis in both normally hydrated and dehydrated type 2 diabetes mice[40]. However, since metformin and the pharmacological activators have other effects besides activation of AMPK, whether AMPK inhibits tonicity-dependent activation of NFAT5 needs to be confirmed with a specific way of manipulating the kinase.

Summary and perspective

NFAT5 is clearly critical for kidney functions. Emerging evidence has shown that its dis-regulation results in or is associated with the renal diseases and disorders. Figure 1 summarizes currently known protein and lipid kinases that involve in regulation of tonicity-dependent activation of NFAT5. More are expected to come. Cells need these kinases working together to orchestrate a specific signal to NFAT5 in response to hypertonic or hypotonic perturbation. These kinases could fulfill their assignments by their different activation duration and strength, and their network with each other as well as with other signaling molecules and scaffolds in a

specific subcellular location and time. Further work is needed to provide direct pieces of evidence to support this hypothesis. A vast majority of these kinases were identified in cultured cells. They need to be tested directly in the kidney to determine whether they have the same functions *in vivo*. Inhibition of NFAT5 results in the urinary concentration defect, indicative of a decrease in the renal medullary interstitial tonicity[5]. The decrease of the renal medullary tonicity inhibits NFAT5 activity[139]. It is difficult to dissect whether the effect of knockout of a kinase, even when it is done in the kidney epithelium-specific manner, on NFAT5 is from the direct effect on the transcription factor or from an indirect effect secondary to alteration of tonicity in the renal medullary interstitium. This challenge calls for a new technology to address how NFAT5 is regulated in the kidney medulla.

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REVIEW

Water, electrolytes, and acid-base alterations in human immunodeficiency virus infected patients

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Abstract

The clinical spectrum of human immunodeficiency virus (HIV) infection associated disease has changed significantly over the past decade, mainly due to the wide availability and improvement of combination antiretroviral therapy regiments. Serious complications associated with profound immunodeficiency are nowadays fortunately rare in patients with adequate access to care and treatment. However, HIV infected patients, and particularly those with acquired immune deficiency syndrome, are predisposed to a host of different water, electrolyte, and acid-base disorders (sometimes with opposite characteristics), since they have a modified renal physiology (reduced free water clearance, and relatively increased fractional excretion of calcium and magnesium) and they are also exposed to infectious, inflammatory, endocrinological, oncological variables which promote clinical conditions (such as fever, tachypnea, vomiting, diarrhea, polyuria, and delirium), and may require a variety of medical interventions (antiviral medication, antibiotics, antineoplastic agents), whose combination predispose them to undermine their homeostatic capability. As many of these disturbances may remain clinically silent until reaching an advanced condition, high awareness is advisable, particularly in patients with late diagnosis, concomitant inflammatory conditions and opportunistic diseases. These disorders contribute to both morbidity and mortality in HIV infected patients.

Key words: Human immunodeficiency virus; Acquired immune deficiency syndrome; Salt; Water; Potassium; Acid-base

Core tip: Human immunodeficiency virus infected patients, and particularly those with acquired immune

deficiency syndrome, are predisposed to different water, electrolyte, and acid-base disorders since they have a modified renal physiology and they also are exposed to infectious, inflammatory, endocrinological, oncological, and pharmacological variables whose combination undermine their homeostatic capability. We herein discuss each of these internal milieu alterations usually observed in this group.

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INTRODUCTION

The clinical spectrum of human immunodeficiency virus (HIV) infection associated disease has changed significantly over the past decade, mainly due to the wide availability and improvement of combination antiretroviral therapy regiments. Serious complications associated with profound immunodeficiency are nowadays fortunately rare in patients with adequate access to care and treatment. Currently, most complications observed in patients with HIV infection are derived from serious but non-acquired immune deficiency syndrome (AIDS) defining clinical events, which are more frequent due to the chronic inflammatory status promoted by the virus itself and are further aggravated by the use of some antiretroviral agents[1,2].

Renal disorders have been increasingly reported in the context of human retroviral infection, particularly the decrease over time of estimated glomerular filtration rate (eGFR), nephrotic syndrome, and proximal tubular deficiency associated with the use of tenofovir disoproxil-fumarate (TDF) and some protease inhibitors such as lopinavir/ritonavir and atazanavir[3]. Although periodic evaluation of renal function (*e.g.,* serum creatinine, eGFR) and proteinuria are routinely recommended in the care of these patients, much less is known or published about specific renal water handling abnormalities, electrolyte disturbances and alterations of acid-base balance in patients with HIV infection[3-5].

HIV infected patients, in particular those with advanced disease may be affected by infectious, autoimmune and oncologic diseases that promote clinical conditions (such as fever, tachypnea, vomiting, diarrhea, polyuria, and delirium), and may require a variety of medical interventions (antiviral medication, antibiotics, antineoplastic agents), whose combination predispose them to develop different sort of electrolytes disorders[6,7].

In the present report, renal water, electrolyte, and acid-base disorders in the HIV infected patients are analyzed.

RENAL WATER AND ELECTROLYTES HANDLING IN HIV PATIENTS

Renal water and electrolytes handling in HIV patients on different therapeutic regimens including with tenofovir, with non-tenofovir, and without antiretroviral drugs (naïve). These exploratory renal physiology studies (urine concentration and dilution tests) found no significant differences in sodium, potassium, chloride, phosphorus, calcium, magnesium, glucose, urea, and uric acid renal handling between healthy volunteers and stable HIV patients with normal renal function, independently whether or not they were receiving anti-retroviral therapy. However, a significant reduction in maximal urine concentration - dilution capability in stable HIV patients compared to healthy volunteers was consistently documented. In this study maximum free water clearance showed values three times lower in HIV patients than in the healthy volunteers despite normal osmolar clearance[8,9]. This finding may explain the reason why HIV patients were slightly hyponatremic during the dilution test. The urine concentration-dilution defect was attributed to a dysfunction in the thick ascending limb of the loop of Henle (TAHL). Since, HIV has been detected in renal tubular cells, this suggests that either the infection itself or the associated inflammatory process may produce direct tubular damage which appears to be independent of the presence of antiretroviral treatment. This finding also means that there may be an increased risk of developing hyponatremia in stable HIV-infected patients who undergo a water load or receive hyponatremia inducing drugs, as well as dehydration when they are exposed to settings of water loss and impaired thirst or intake of water[8-10]. A recent study has shown that in a setting of volume expansion, where tubule reabsorption is reduced because of the high urinary flux, there was a significant reduction in serum calcium and magnesium values, as well as a concomitant and significant increase in their urinary fractional excretion in stable HIV-positive patients compared to healthy volunteers[9]. Since calcium and magnesium are importantly reabsorbed in TAHL, and this segment show dysfunction in this population, a basal TAHL reabsorption defect worsened by the increased urinary flux was suggested. This finding means that there is an increased risk for developing hypocalcemia or hypomagnesemia in stable HIV-infected patients who undergo volume expansion or who receive hypocalcemia or hypomagnesemia-inducing drugs[9-11].

SALT AND WATER BALANCE IN HIV INFECTION AND AIDS

Salt and water imbalances can induce abnormalities in extra-cellular volume status and/or serum sodium depending on the nature of this alteration (increase or decrease), its absolute magnitude (mild or severe), and

its relative magnitude (body sodium content relative to body water content)[12]. A significant salt and water depletion generates real hypovolemia, and if this depletion involves an excess of hypotonic fluid loss, it can generate hypernatremia (serum sodium > 145 mmol/L), while if the loss of salt is in excess of water it may generate hyponatremia (serum sodium < 135 mmol/L)[12].

Salt and water retention induces an increase in ECF that, depending on its pathophysiologic mechanism, it may appear either as hypervolemia and edema (*e.g.*, renal failure) or effective arterial hypovolemia and edema (*e.g.*, cirrhosis, cardiac failure, some of nephrotic syndromes). Another factor that can modify the sodium/water ratio is body potassium content since its intracellular depletion induces hyponatremia by at least two mechanisms: A shift of sodium to the intracellular space, and possibly by aberrant vasopressin release. Edelman summarized these concepts in the following equation[13]: Serum sodium = [body (exchangeable) sodium content + body (exchangeable) potassium content]/total body water content.

Additionally, there are two infrequent causes of hyponatremia: First, a hyponatremia secondary to an overtly excessive water intake which overcomes renal capability of free water excretion and is associated with fully suppressed vasopressin secretion, especially in states of low osmolar excretion. This type of hyponatremia has been documented in AIDS patients who suffered from dementia and primary polydipsia[14]. Second, a reset osmostat hyponatremia, usually found in malnourished chronically-ill AIDS patients[12]. Based on the above mentioned pathophysiological mechanisms, hyponatremia is currently classified depending on patient's plasma tonicity level into: Hypertonic, normotonic, or hypotonic hyponatremia. In addition, hypotonic hyponatremia is classified depending on patient's extracellular fluid (ECF) status with low, normal or high ECF[12]. Each type of hyponatremia in AIDS patients was described as follows:

HYPONATREMIA

Normotonic hyponatremia

Normotonic hyponatremia or pseudohyponatremia (PSH) consist of a low serum sodium value in a context of normal plasma tonicity, since it is a measurement artifact caused by an increase in the solid fraction of plasma, usually due to hyperlipidemia or hyperproteinemia[4]. A direct ion-sensitive electrode potentiometry-based estimation can avoid this error[15]. Also, the addition of a non-electrolyte osmoles (sorbitol, manitol, sucrose) to the extra-cellular space with redistribution of sodium-deficient water from the intracellular space can cause this finding[14]. PSH has been described in HIV patients who have important hypergammaglobulinemia which may be related to disease progression or its response to antiretroviral therapy. Besides, polyclonal hypergammaglobulinemia in this population it may also be secondary to a co-infection with hepatitis C. It is important to identify PSH since treating it as hypotonic hyponatremia can cause severe dehydration and even death[14,16].

Hypertonic hyponatremia

Since in absence of renal failure, plasma osmolality (Posm) is mainly determined by serum sodium and glucose level (Calculated Posm = serum sodium × 2 + glycemia/18 + uremia/6), hypertonic hyponatremia is observed in hypertonic variety of uncontrolled diabetes mellitus with severe hyperglycemia. Hyperglycemia increases extracellular tonicity which extracts sodium-deficient water out of the intracellular space diluting the serum sodium concentration in the extracellular space, inducing hyponatremia[12]. Other solutes, like sorbitol and manitol can behave similarly.

Hypotonic hyponatremia

Patients with cardiac, hepatic, renal, lung, intracranial, and endocrine diseases can develop hypotonic hyponatremia secondary to an excess of water consumed voluntarily or administered iatrogenically, when urine free water excretion is impaired due to a decreased circulatory delivery of fluid to diluting segments (cardiac failure), altered TALH segment function (tubulopathy), and/or (inappropriate or appropiate) vasopressin release[12].

Since impairment of the function of the afore mentioned organs is frequent in the context of AIDS and associated complications, hyponatremia is not surprisingly the most frequent electrolyte abnormality (23.5%-75%) seen both in non-hospitalized and hospitalized patients with HIV infection and AIDS[17-20]. Hyponatremic patients with AIDS are more prone to morbidity and mortality and frequently manifest complicating opportunistic infection-related illnesses (particularly Pneumocystis jiroveci and cytomegalovirus)[19]. However, this poor prognosis has not been attributed to this electrolyte disorder since most of the patients were normonatremic at death, and their higher mortality has been attributed to the severity of their immune-compromised state: For instance, severe hyponatremia (serum sodium < 125 mmol/L) was associated to a lower CD4 T cell count than in AIDS patients who did not have hyponatremia[8,17,21,22].

Dao *et al*[23] also reported a higher mortality rate among women who showed hyponatremia and hypochloremia (in that context it means a serum chloride value significantly lower respect to the expected one for hyponatremia) compared with women who only had one electrolyte abnormality. This observation suggests that a combination of both disorders (hyponatremia + hypochloremia) may suggest a more profound clinical disturbance in a HIV patient, such as the one secondary to subclinical tuberculosis or cryptococcal lung or cerebral infection. Each type of hypotonic hyponatremia in AIDS patients has been described as follows:

Table 1 Causes of hypotonic hyponatremia in human immunodeficiency virus infected patients

Hyponatremia with normal ECF
SIADH: Lungs or central nervous system infection or neoplasm
Hypothyroidism: Low T3 syndrome, pituitary infections, thyroiditis and miconazole
Glucocorticoid deficiency: Glucocorticoid axis damaged
Hyponatremia with low ECF (volume depletion)
Digestive losses: vomiting, diarrhea
Renal losses: CSW, interstitial nephritis, cortisol resistance and adrenal insufficiency
Hyponatremia with high ECF (edematous states)
Non-renal causes: cirrhosis, heart failure
Renal causes: acute tubular necrosis, intra-tubular obstruction, interstitial nephritis, nephrocalcinosis, hemolytic-uremic syndrome, collapsing focal and segmental glomerulosclerosis
Hyponatremia secondary to drugs
Renal insufficiency
Interstitial nephritis
Impair maximal urinary dilution capability by direct tubular effect
Cortisol deficiency
SIADH effect

ECF: Extracellular fluid; SIADH: Syndrome of inappropriate antidiuretic hormone release; CSW: Cerebral salt wasting.

Hyponatremia with normal ECF (Table 1)

Syndrome of inappropriate antidiuretic hormone release: This is an entity induced by free water retention secondary to an inappropriate (for the level of serum osmolality) vasopressin hormone release or an excessive response of its receptor (V2 receptor) in the collecting tubules, in the context of normal GFR, normal thyroid and adrenal gland function, and in the absence of hyponatremia inducing drugs[12,24].

Syndrome of inappropriate antidiuretic hormone release (SIADH) may be present in up to 36% of patients with advanced HIV infection and it can by induced by infection (neurosyphilis, *etc.*) neoplasm of the lungs or central nervous system[8,25]. SIADH must be differentiated (not always easy) from cerebral salt wasting syndrome (CSW) since both entities can appear in AIDS patients, and may present as hyponatremia with high urinary sodium, and elevated circulating natriuretic peptide and vasopressin levels. However, CSW patients show clinical signs of hypovolemia, increased serum urea:creatinine ratio, normal or high serum uric acid, lower fractional excretion of uric acid, and very high urinary sodium levels, while SIADH patients show slight hypervolemia, low urea: Creatinine ratio, low serum uric acid, higher fractional excretion of uric acid, and high urinary sodium levels[12,26].

Central pontine myelinolysis, a severe neurological disease that may be observed in hyponatremia and its overly rapid correction, has also been documented in patients with AIDS, particularly in those with advanced HIV infection, prolonged hyponatremia, anorexia, hypoalbuminemia, chronic alcoholism, disseminated malignancy, and in those patients treated with systemic chemotherapy. The clinical presentation varies between rapidly evolving spastic paraparesis with pseudobulbar palsy, and changes in mental state such as confusion or coma[26-28].

Hyponatremia secondary to hypothyroidism: Several hyponatremia-inducing mechanisms have been described in patients suffering from hypothyroidism, such as reduced function of the nephron diluting segment due to low renal perfusion secondary to decrease cardiac output, inappropriate vasopressin secretion, and increased urinary salt loss[29-36].

The most frequent cause of hypothyroidism in AIDS patients is the "low T3 syndrome" which shows a normal thyroid production of T3, but an impaired peripheral conversion of T4 to T3, since 80% of serum T3 usually comes from T4 deodination in peripheral tissues. Another cause of reduction in thyroid function in this population is centrally-induced hypothyroidism secondary to pituitary infections caused by Pneumocystis, cytomegalovirus, toxoplasmosis, neurosyphilis, and HIV itself; or decrease in hypothalamic thyrotropin releasing hormone due to the wasting syndrome induced by AIDS (non-thyroidal illness syndrome)[25,36]. Finally, hypothyroidism secondary to Hashimoto's thyroiditis (autoimmunity induced by increased B cell activation), and antifungal agents such as miconazole have been described[36,37].

Hyponatremia secondary to glucocorticoid deficiency: Since cortisol exerts a negative effect on neurophysiological vasopressin secretion, its deficit can promote an inappropriate vasopressin release, and consequently an increased trend to hyponatremia[37]. The isolated cortisol deficit can be generated by any infectious, immunologic, or oncologic damage in the glucocorticoid axis[38,39].

Hyponatremia with low ECF

The most common cause of hyponatremia in the AIDS population is one caused by volume depletion secondary to vomiting, diarrhea, or tubular disorders[8]. Volume depletion can induce hyponatremia by stimulating the non-osmotic vasopressin release, an appropriate response for protecting the intravascular volume, in a setting of an adequate or excessive oral water (hypotonic

solution) intake[40]. Sodium losses lead to hypovolemia and consequently induce adequate vasopressin secretion, thus hyponatremia is promoted in this case by a double mechanism: A reduction in body sodium content (sodium loss) and an increase in body water content (water retention). Negative sodium balance is worsened in settings where sodium reabsorption is ineffective, as is the case in CSW, interstitial nephritis, adrenal insufficiency[12] (Table 1).

Gastrointestinal losses: This is the second most common cause of hyponatremia in patients with HIV infection and AIDS, particularly when it is represented by diarrhea (induced by HIV or other organisms) in a setting of low electrolyte content fluid replacement[8,12].

CSW: CSW is an uncommon disorder characterized by hyponatremia, volume depletion and clinical response to water and salt replacement[26]. The etiology of this entity has been attributed to a decrease in the sympathetic nervous system outflow leading to decrease sodium reabsorption in proximal tubules, inhibition of RAAS, and also release of natriuretic peptides (*e.g.*, atrial and brain natriuretic peptides)[26,39]. CSW occurs in patients with a central nervous system insults, and its similarity with SIADH makes crucial its recognition as water restriction, a SIADH-oriented treatment, is detrimental to patients with unrecognized CSW[21,22]. Even though, differentiation between CSW and SIADH is not so simple, CSW tends to be characterized by the presence of clinical hypovolemia, normal or increased serum urea and uric acid levels, and polyuria with much more higher sodium excretion compared to SIADH[39,40,41].

Interstitial nephritis: Interstitial nephritis represents another potential cause of urine loss of salt which can induce volume depletion in AIDS patients since they are exposed to polypharmacy and/or autoimmunity disorders[38].

Adrenal insufficiency: The prevalence of adrenal insufficiency is up to 22% in AIDS patients[42,43]. Both HIV itself as well as concomitant disseminated tuberculosis can cause suppression of hypothalamus pituitary-adrenal axis and destruction of adrenal gland; and this may lead to adrenal insufficiency and subsequent hyponatremia. Other opportunistic organisms that can induce hypoadrenalism in HIV patients are Cytomegalovirus, Cryptococcus neoformans, Mycobacterium avium-intracellulare, Pneumocystis jiroveci, Histoplasma capsulatum, and Blastomyces dermatitidis. Moreover, hypoadrenalism in this population can be induced by adrenal gland damage due to Kaposi's sarcoma, lymphoma, or adrenocortical hemorrhage secondary to a coagulopathy, as well as to pharmacological intervention, as is the case of ketoconazole (inhibition of steroids synthesis), rifampicin, and phenytoin (increased cortisol metabolism)[39-44].

Cortisol resistance: In this entity, patients suffering from advanced HIV infection present clinical features suggestive of hypoadrenalism, such as asthenia, mucocutaneous melanosis, hypovolemic hyponatremia, but serum testing reveal high serum cortisol and normal/high adrenocorticotropic hormone levels. This particular clinical setting of cortisol resistance characteristically improves with high doses of glucocorticoids[45-47]. In this case the presence of hyperkalemia with low potassium excretion can help to differentiate this entity from CSW[38].

Hyponatremia with high ECF

This sort of hyponatemia is observed in severe edematous states secondary to cardiac, hepatic or renal insufficiency, as well as uncommonly in severe nephrotic syndrome.

In clinical settings of effective hypovolemia such as severe cardiac or hepatic insufficiency, and some nephrotic syndromes, hypotonic hyponatremia appears as a consequence of an impaired circulatory delivery to diluting segments, in combination with adequate vasopressin release (effective hypovolemia)[12]. On the other hand, in clinical settings of hypervolemia such as severe renal insufficiency, hypotonic hyponatremia appears as a consequence of an impair capability of free water excretion due to a significantly decrease in GFR (lower than 5 mL/min per 1.73 m^2)[12].

Renal insufficiency is a well-known complication in HIV positive patients, usually induced by a heterogeneous collection of miscellaneous mechanisms: Acute tubular necrosis (toxic, ischemic), intra-tubular obstruction from uric acid or phosphate (tumor destruction), different type of glomerular (glomerulonephritis, *etc.*), tubulointerstitial (interstitial nephritis, nephrocalcinosis, *etc.*), and vascular diseases (atypical hemolytic-uremic syndrome), and also a particular type of focal and segmental glomerulosclerosis only found in this population called HIV associated nephropathy often of a collapsing variant[8,22,26,48] (Table 1).

Hyponatremia secondary to drugs

Drug induced hyponatremia is the third most common cause of hyponatremia in AIDS patients[8].

Medication can induce hyponatremia by different mechanisms, and therefore this type of hyponatremia described here separately. AIDS patients may frequently receive medications that can induce hyponatremia by promoting water retention and/or sodium loss[17,49,50]: (1) renal insufficiency (co-trimoxazole); (2) interstitial nephritis (trimethoprim, loop diuretics, thiazides); (3) impair maximal urinary dilution capability by direct tubular effect (thiazides); (4) cortisol deficiency (rifampin, ketoconazol, suramin); (5) SIADH effect (pyrazinamide, ethambutol, narcotics, lopinavir, ritonavir); and (6) undefined mechanism (amphotericin B, pentamidine) (Table 1).

Table 2 Causes of hypernatremia in human immunodeficiency virus infected patients

Hypernatremia
Increased insensible water losses: Fever and tachypnea
Increased digestive water losses: Vomiting, diarrhea
Increased urinary water losses: Central diabetes insipidus, nephrogenic diabetes insipidus secondary to nephrocalcinosis or tubule-interstitial damage caused by infection, tumors, drugs
Reduced water intake: Unconsciousness, adipsia: Thirst´s center destruction by a vascular, neoplastic or infectious cause

HYPERNATREMIA

This disorder occurs when a large loss of free water is combined with an inadequate amount of water ingestion or insufficient iatrogenic provision of water in unconscious patients, and it was reported in up to 31% of patients with very advanced disease[49-51] Among the main causes of free water loss in AIDS patients are[26,48]: (1) fever with insensible water losses through the lung and skin; (2) digestive water losses: Vomiting, diarrhea; (3) central diabetes insipidus secondary to toxoplasmosis or cytomegalovirus encephalitis; and (4) nephrogenic diabetes insipidus secondary to nephrocalcinosis, tubule-interstitial diseases caused by infections (cytomegalovirus, Mycobacterium avium intracellulare, systemic mycoses), tumors (lymphoma), or medication, such as rifampin, foscarnet, and amphotericin B.

Regarding hypernatremia secondary to low water intake in AIDS patients, it has been described in unconscious patients affected by a neurological disorder, or in those patients suffering from adipsia. The latter is a rare hypothalamic condition in which a conscious patient develops serum hyperosmolality secondary to reduced water intake because he/she have no thirst. This disorder commonly is associated with lack of vasopressin release, which was attributed to vascular, neoplastic, or granulomatous destruction of the osmoreceptor and thirst center[52] (Table 2).

POTASSIUM IMBALANCE IN AIDS

Potassium is the main cation in the intracellular space, its total body content in healthy adults is around 3700 mmol, and muscle tissues represent its main body reserve. Potassium has two significant balances: The external balance between the organism and the environment, and the internal balance between the intracellular compartment and the extracellular compartment within the organism[53-55]. The external balance depends on nutrition as well as colonic (20%) and renal (80%) potassium excretion, and this excretion depends both on GFR and potassium distal tubule secretion, which is mainly stimulated by aldosterone hormone. The internal balance depends on the potassium shifts between intracellular and extracellular compartments. Insulin and the adrenergic system are the main stimuli for its intracellular shift along with metabolic alkalosis, plasma hypotonicity and beta-adrenergic sympathetic tone, while the main stimuli for its extracellular shift are glucagon, metabolic acidosis, plasma hypertonicity, and alpha-adrenergic sympathetic tone[53-56].

Hypokalemia

Hypokalemia (serum potassium < 3.5 mmol/L) has been reported in about 19% of patients with AIDS[54]. The main causes of hypokalemia in this population are gastrointestinal potassium losses, usually induced by profuse diarrhea secondary to intestinal infection, intestinal tumor, or AIDS-associated enteropathy[8,57]. Vomiting is another important cause of hypokalemia, not only by direct potassium loss (emesis) but also increasing urinary potassium excretion by inducing hypovolemia, bicarbonaturia and consequently secondary hyperaldosteronism[55]. Urinary potassium wasting can also accompany tubule injury secondary to direct toxic effect of nephrotoxic drugs (*e.g.*, amphotericin B, aminoglycosides) or interstitial nephritis or secondary to some antibiotics (*e.g.*, sulfonamides, cephalosporins) or non-steroidal anti-inflammatory (NSAIDs) drugs[56-62]. Anorexia and low potassium intake, sarcopenia and myopathy (low potassium body reserves) observed in HIV-associated wasting syndrome exacerbate the risk of hypokalemia in this population[8,63]. In addition, acquired tubulopathies can also induce urinary electrolytes wasting, and as a consequence hypomagnesemia, hypocalcemia, and hypophosphatemia (Fanconi syndrome) can develop in this population[64-70]. Among the main tubulopathy inducing drugs in AIDS patients are: TDF, foscarnet, zidovudine and didanosine[67-72] (Table 3).

Hyperkalemia

Hyperkalemia (serum potassium > 5.5 mmol/L) has been reported in 5%-53% of AIDS patients[55,73]. Two main mechanisms of hyperkalemia have been described in these patients. First, reduced urinary potassium excretion (external balance), such as the one observed with severe renal failure (GFR < 5 mL/min per 1.73 m²), hyperkalemia inducing drugs (ACEIs, NSAIDs, trimethoprim), adrenal insufficiency, and hyporeninemic hypoaldosteronism[21,39-43,51,74-77]. Second, increased shift of potassium from the intracellular compartment to the extracellular compartment (internal balance), such as, rhabdomyolysis, tumor lysis syndrome after chemotherapy in AIDS patients affected by malignancies, and diabetes mellitus[77-80]. In this case plasma hypertonicity induced by severe hyperglycemia, develop hyperkalemia through osmotically induced water and potassium shifts from the intracellular compartment to

Table 3 Causes of dyskalemia in human immunodeficiency virus infected patients

Hypokalemia
Increased gastrointestinal K^+ losses: Diarrhea: Infection, tumor or AIDS-associated enteropathy
Increased urinary K^+ losses: Vomits, tubule toxicity, interstitial nephritis
Low K^+ body content: Low potassium intake, sarcopenia and myopathy
Hyperkalemia
Reduced urinary K^+ excretion: Drugs, adrenal insufficiency, hyporeninemic hypoaldosteronism
Increased K^+ shift to EC: Rhabdomyolysis, tumor lysis syndrome, hyperglucemia

K^+: Potassium; EC: Extracellular compartment; AIDS: Acquired immune deficiency syndrome.

Table 4 Causes of acid-Base disorders in human immunodeficiency virus infected patients

Acidosis
Hyperchloremic metabolic acidosis: Diarrhea, tubular damage secondary to drugs, hypergammaglobulinaemia, acute tubular necrosis, interstitial nephritis, HIV
High anion gap metabolic acidosis: Uremia, diabetic ketoacidosis, lactic acidosis (type A or B)
Alkalosis
Metabolic alkalosis (volume contraction): Gastro-intestinal losses, urinary losses
Respiratory alkalosis (hyperventilation): Central nervous system alteration, altered liver function, lung opportunistic infections and malignancies

HIV: Human immunodeficiency virus.

the extracellular (intravascular) compartment[12] (Table 3).

ACID-BASE DISORDERS

Acid-base imbalance generates different sort of internal milieu disorders such as acidosis, alkalosis, or their combination (double or triple acid-base disorders). Acidosis is the pathophysiologic process characterized by either a primary acid gain or a primary alkali loss, while acedemia indicates an increased H^+ concentration in the blood (blood pH < 7.36). Conversely, alkalosis is the pathophysiologic process characterized by either a primary acid loss or primary alkali gain, and alkalemia indicates a decreased H^+ concentration in the blood (blood pH > 7.44). Additionally, acidosis is usually classified depending on its pathophysiologic mechanism in respiratory acidosis (carbon dioxide retention), normochloremic or high anion-gap metabolic acidosis (bicarbonate conversion), and hyperchloremic or normal anion-gap metabolic acidosis (bicarbonate loss). On the other hand, alkalosis is usually classified depending on their pathophysiologic mechanism in respiratory alkalosis (carbon dioxide high excretion) and metabolic alkalosis (bicarbonate gain)[81].

In AIDS patients the main cause of hyperchloremic (normal anion-gap) metabolic acidosis is bicarbonate loss through profuse diarrhea or renal tubule dysfunction induced by drugs (TDF, pentamidine, amphotericin, B, rifampicin, ethambutol, cidofovir, adefovir, abacavir or nelfinavir), hypergammaglobulinaemia, renal diseases (acute tubular necrosis, atopic or infectious interstitial nephritis), adrenal insufficiency (type Ⅳ distal tubule acidosis), and even HIV direct tubular cytopathic effect[11,51,56,74,75,82-91].

On the other hand, normochloremic (high anion-gap) metabolic acidosis has been documented in AIDS during severe renal failure (uremic acidosis), diabetic acidosis (ketoacidosis) secondary to pentamidine-induced pancreatic damage, and in sepsis, systemic inflammatory response syndrome, or non-Hodgkin lymphoma (hypoxic lactic acidosis: Type A)[8,22,26,82,83]. It is worth mentioning that lactic acidosis secondary to lymphoma is considered a paraneoplastic syndrome of poor prognosis, since lactate production increases as the aggressive tumor outgrows its blood supply resulting in local hypoxia in the absence of any systemic hypoxia or hypoperfusion. As pathophysiological mechanism an increased glycolytic activity causing an increase in lactic acid generation, overexpression of the glycolytic enzyme hexokinase Ⅱ or increased IGF-binding protein activity, has been proposed[83].

A particular type of non-hypoxic lactic acidosis (type B) has been described with the use of antiretroviral drugs that are no longer recommended, such as zalcitabine, stavudine, didanosine or zidobudine. This entity is explained mainly by mitochondrial toxicity and reveals hyperlactataemia without lactic acidosis to overt life-threatening lactic acidosis[86-97]. These antiretroviral drugs are nucleosidic inhibitors of viral reverse transcriptase which alter mitochondrial function by inhibiting the mitochondrial DNA polymerase gamma (the enzyme responsible for the replication of mitochondrial DNA). The diminution in this DNA content provokes a diminished synthesis of respiratory chain enzymes[94]. Metabolic alkalosis is frequently induced in these patients by volume contraction secondary to gastrointestinal (vomiting, diarrhea) or urinary losses (diuretics, polyuria, *etc.*)[8,84-86,90]. Opportunistic infections (*e.g.*, histoplasmosis, *etc.*) and malignancies affecting the respiratory tract, the central nervous system, or/and liver function can stimulate hyperventilation and as a

consequence induce respiratory alkalosis[98,99] (Table 4).

CONCLUSION

HIV infected patients, and particularly those with AIDS, are predisposed to a host of different water, electrolyte, and acid-base disorders (sometimes with opposing effects), since they are exposed to infectious, inflammatory, oncological, and pharmacological variables whose combination undermine their homeostatic capability. As many of these disturbances may remain clinically silent until reaching an advanced condition, high awareness is advisable, particularly in patients with late diagnosis, concomitant inflammatory conditions and opportunistic diseases. These disorders contribute to both morbidity and mortality in HIV infected patients.

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REVIEW

Adult stem cells as a tool for kidney regeneration

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Abstract

Kidney regeneration is a challenging but promising strategy aimed at reducing the progression to end-stage renal disease (ESRD) and improving the quality of life of patients with ESRD. Adult stem cells are multipotent stem cells that reside in various tissues, such as bone marrow and adipose tissue. Although intensive studies to isolate kidney stem/progenitor cells from the adult kidney have been performed, it remains controversial whether stem/progenitor cells actually exist in the mammalian adult kidney. The efficacy of mesenchymal stem cells (MSCs) in the recovery of kidney function has been demonstrated in animal nephropathy models, such as acute tubular injury, glomerulonephritis, renal artery stenosis, and remnant kidney. However, their beneficial effects seem to be mediated largely *via* their paracrine effects rather than their direct differentiation into renal parenchymal cells. MSCs not only secrete bioactive molecules directly into the circulation, but they also release various molecules, such as proteins, mRNA, and microRNA, in membrane-covered vesicles. A detailed analysis of these molecules and an exploration of the optimal combination of these molecules will enable the treatment of patients with kidney disease without using stem cells. Another option for the treatment of patients with kidney disease using adult somatic cells is a direct/indirect reprogramming of adult somatic cells into kidney stem/progenitor cells. Although many hurdles still need to be overcome, this strategy will enable bona fide kidney regeneration rather than kidney repair using remnant renal parenchymal cells.

Key words: Adult stem cells; Direct reprogramming; Extracellular vesicles; Mesenchymal stem cells; Paracrine factors; Indirect reprogramming

Core tip: Although intensive studies have been performed to isolate kidney stem/progenitor cells from the mammalian adult kidney, whether stem/progenitor cells actually exist in the adult kidney is still debated. Mesenchymal stem cells seem to exert beneficial effects *via* paracrine effects rather than by direct differentiation into renal parenchymal cells. In this review, we also introduce potential roles of extracellular vesicles released

from stem cells and direct/indirect reprogramming of adult somatic cells by which kidney stem/progenitor cells will be formed in the future.

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INTRODUCTION

The kidney is a vital organ that plays various roles, such as the excretion of waste products; regulation of systemic fluid volume, electrolytes, and pH; maintenance of systemic blood pressure; and erythropoietin production. These functions are performed by the nephrons, the functional units of the kidney. If the structure and/or function of the nephrons are damaged because of diseases, such as diabetes, hypertension, and glomerulonephritis, and if such damages continue to progress, renal function gradually deteriorates. The kidney finally becomes unable to perform its critical roles, resulting in renal failure.

There are two therapeutic options for the treatment of end-stage renal disease (ESRD). One is dialysis therapy, which compromises patients' quality of life and cannot substitute for all kidney functions. Another is renal transplantation, which is limited because of the lack of sufficient donors. To explore a better treatment for ESRD, it is necessary to find strategies to regenerate the kidney. In this respect, stem cell therapy for the kidney has been intensively studied recently.

Stem cells are defined as cells that are capable of self-renewal and can differentiate into a variety of phenotypes[1]. Adult stem cells (ASCs) are multipotent stem cells that reside in various tissues, such as the bone marrow, adipose tissue, and skeletal muscle[2,3]. In this article, we review the possibility of kidney regeneration using ASCs.

Stem cells in the embryonic kidney

Although stem/progenitor cells in the embryonic kidney are beyond the scope of this review, we briefly describe the process of kidney organogenesis, because genetic programs that are activated during kidney organogenesis are reactivated in disease states, such as acute tubular injuries. Kidney organogenesis initiates with the interaction of the ureteric bud (UB) derived from the Wolffian duct with the metanephric mesenchyme (MM). A proportion of the MM is located adjacent to the UB, called the cap mesenchyme (CM). The CM then aggregates at the tip of the UB and differentiates into all epithelial cells of nephrons, except the collecting tubules. It is now well established that the CM contains stem/progenitor cells for kidney organogenesis[4]. The CM expresses unique transcription factors, such as *Pax*2, *Six*2, and *Sall*1[5]. Although stem cells in the embryonic kidney are a promising source for kidney regeneration, their clinical use is strictly limited mainly because of the ethical concerns and small number of stem cells. Therefore, the search for stem/progenitor cells in the adult kidney has been intensively performed.

Stem/progenitor cells in the adult kidney

Four different methods have been used in an attempt to isolate stem/progenitor cells from the adult kidney (Table 1).

Label-retaining cells (LRCs): To conserve the proliferation capacity for a lifetime and to prevent genetic injuries during mitosis, stem cells cycle very slowly. Stem/progenitor cells in the kidney were isolated using this property. Cells were pulse-labeled with a dye, such as 5-bromo-2-deoxyuridine. Then, slow-cycling LRCs were detected following a chase period. Maeshima *et al*[6] detected LRCs predominantly in the renal tubular cells of the adult rat kidney. LRCs proliferated in response to ischemia/reperfusion injury and contributed to the repair of renal tubules. In another study, Maeshima *et al*[7] also demonstrated that LRCs were integrated into epithelial components of the nephron when transplanted into the metanephric kidney, suggesting that LRCs were multipotent stem cells. Oliver *et al*[8] detected LRCs in the kidney papilla of adult rats. LRCs proliferated after the induction of ischemia in the kidney and migrated toward the medulla. They also injected renal papillary cells into the subcapsular area of the kidney and found that some cells were incorporated into renal tubules.

Side population cells: Because stem cells extrude dyes, such as Rhodamine 123 and Hoechst 33342, *via* the ATP-binding cassette protein[9], they are located in a unique position on the fluorescent-assisted cell sorting scattered plot and are called side population (SP) cells. Iwatani *et al*[10] isolated SP cells from the adult rat kidney. However, the cells did not participate in the kidney repair following experimental glomerulonephritis or gentamicin-induced nephropathy. Hishikawa *et al*[11] isolated SP cells from the adult murine kidney. These cells expressed musclin/MyoR and improved renal function when injected systemically into mice with the induction of acute tubular injury by cisplatin administration. Furthermore, SP cells expressed renoprotective factors, such as hepatocyte growth factor (HGF), vascular endothelial growth factor, and bone morphogenetic protein 7 in a cisplatin-induced acute kidney injury (AKI) model. Challen *et al*[12] also isolated SP cells from the adult murine kidney. These cells were located predominantly in the proximal tubules and integrated into the MM- and UB-derived structures when injected into the embryonic kidney, suggesting that they were multipotent stem cells. However, these cells were barely incorporated into the renal tissues when

Table 1 A summary of the results of isolating stem cells from the adult kidney

Species	Isolation method	Stem cell markers	Location	Incorporation into kidney tubules	Ref.
Rat	Label retaining		Proximal tubule	Yes	Maeshima *et al*[6]
Rat	Label retaining		Papilla	Yes	Oliver *et al*[8]
Rat	Side population	Sca-1, CD45	Some were derived from bone marrow	No	Iwatani *et al*[10]
Mouse	Side population	Sca-1	Interstitium	NE	Hishikawa *et al*[11]
Mouse	Side population	Sca-1	Proximal tubule	Yes (rare)	Challen *et al*[12]
Human	Marker	CD133	Tubules	Yes	Bussolati *et al*[15]
Human	Marker	CD133, CD24	Parietal epithelium in the Bowman's capsule	Yes	Sagrinati *et al*[16]
Mouse	Marker	Sca-1	Papilla	Yes	Dekel *et al*[17]
Rat	Culture	Pax2, Oct4	Proximal tubule	Yes	Gupta *et al*[18]

NE: Not examined.

administered to an adriamycin-induced kidney injury model, although renal function was recovered, probably because of their paracrine effect.

Cell surface markers: Cell surface markers, such as CD133, were used to isolate stem/progenitor cells from the adult kidney. Although CD133 is not a specific marker for kidney stem cells, it is a universal marker for stem cells in other tissues, such as hematopoietic stem cells, vascular endothelial progenitor cells (EPCs), and cancer stem cells[13,14]. Bussolati *et al*[15] isolated CD133+ cells from the adult human kidney. These cells expressed *Pax*2, but not CD34 or CD45, markers for hematopoietic stem cells. They could also be induced to differentiate into tubular epithelial cells and endothelial cells *in vitro*. When these cells were administered intravenously in a glycerol-induced AKI model of severe combined immune deficiency (SCID) mice, they were incorporated predominantly into the proximal and distal tubules. Sagrinati *et al*[16] isolated CD133+ and CD24+ cells from human parietal epithelial cells in the Bowman's capsule after culturing glomeruli. When cultured in appropriate conditions, these cells were differentiated into tubular epithelial cells, osteogenic cells, adipocytes, and neuronal cells. These cells were integrated predominantly into renal tubules when they were injected intravenously in SCID mice that were treated with glycerol to induce acute tubular injuries. Dekel *et al*[17] isolated stem cell antigen-1 (Sca1)-positive cells from the adult mouse kidney. The Sca1+ cells were located mainly in the papilla. When these cells were administered to an ischemia-induced AKI model, some of these cells were integrated into renal tubules.

Cell culture: A unique cell population was isolated during the culture of dispersed cells derived from the adult kidney. Gupta *et al*[18] isolated progenitor-like cells from the adult rat kidney that express vimentin, CD90, *Pax*2, and *Oct*4, a marker for embryonic stem cells. These cells were incorporated into renal tubules when injected under the capsule of the kidney or intra-arterially, following ischemia-reperfusion injury of the kidney.

Arguments against the presence of stem/progenitor cells in the mammalian adult kidney

Although aforementioned results suggest that renal stem/progenitor cells exist in the adult kidney, some reports demonstrated that differentiated renal tubular cells, but not renal stem/progenitor cells, can completely regenerate renal tubules after injury. Humphreys *et al*[19] used genetic fate-mapping techniques in which renal epithelial cells derived from the CM (from the Bowman's capsule to the junction of the connecting segment and collecting duct) were labeled with either β-galactosidase or red fluorescent protein (RFP). After ischemia-reperfusion injury, approximately 50% of the proximal tubular cells coexpressed both RFP and Ki67, a cell proliferation marker that is expressed during the S-M phases of the cell cycle. These findings suggested that intrinsic renal tubular cells proliferate in response to injury. Furthermore, approximately 95% of tubular epithelial cells expressed RFP prior to injury, after one cycle of injury, and after two cycles of injury, indicating that no dilution of the RFP+ tubular epithelial cells occurred. These results suggested that differentiated renal epithelial cells proliferate well in response to the injury and that stem and/or progenitor cells residing in the interstitium did not participate in the regeneration of the tubules. Kusaba *et al*[20] used a genetically modified mouse in which the tdTomato protein, which fluoresces in a red color, expressed only in differentiated proximal renal tubules. No dilution of tdTomato+ cells was observed after ischemia-reperfusion injury, suggesting that stem/progenitor cells in renal tubules did not participate in the regeneration of renal tubules. Furthermore, they observed that tdTomate+ proximal tubules expressed CD24 and CD133, markers for stem/progenitor cells. These findings suggested that renal tubules were dedifferentiated and expressed stem cell markers during their proliferation and participation in the repair of renal tubules. These results did not support that stem/progenitor cells in renal tubules and in the interstitium participated in the regeneration of renal

Table 2 Effects of Bone marrow mesenchymal stem cells on renal tissue repair

Origin of stem cells	Experimental model	Effects	Ref.
Mouse	Glycerol-induced AKI	Differentiation into tubular epithelial cells	Herrera *et al*[22]
Mouse	Cisplatin-induced AKI	Differentiation into tubular epithelial cells	Morigi *et al*[23]
Human	Glomerulonephropathy induced by anti-mesangial cell serum	Differentiation into mesangial cells	Wong *et al*[30]
Rat	I/R injury	Recovery of renal function No transdifferentiation into tubules	Lange *et al*[24]
Human	Cisplatin-induced AKI	Recovery of renal function Improved survival No transdifferentiation into tubules	Morigi *et al*[25]
Rat	Gentamicin-induced AKI	Recovery of renal function Conditioned medium and exosomes were effective	Reis *et al*[26]
Rat	Adriamycin-induced nephropathy	Reduced podocyte injury Increased VEGF production	Zoja *et al*[27]
Rat	Thy1.1 GN	Reduced mesangiolysis Increased glomerular cell proliferation Reduced proteinuria Production of VEGF and TGF-β	Kunter *et al*[28]
Mouse	CKD (Deficiency in collagen type IV, α-3 chain)	Reduced fibrosis Production of VEGF and BMP-7	Ninichuk *et al*[29]
Rat	5/6 nephrectomy	Improved renal function Reduced fibrosis Reduced expression of IL-6 and TNF-α	Semedo *et al*[31]
Rat	Kidney allograft	Improved renal function Reduced fibrosis Reduced expression of IL-6	Franquesa *et al*[32]

AKI: Acute kidney injury; I/R: Ischemia reperfusion; GN: Glomerulonephritis; CKD: Chronic kidney disease; VEGF: Vascular endothelial growth factor; TGF-β: Transforming growth factor-β; BMP-7: Bone morphogenetic protein-7; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-α.

tubular cells in acute tubular injury. Nonetheless, a potential role of intrinsic stem/progenitor cells in kidney regeneration is not completely excluded from these results, because stem/progenitor cells may participate in the repair of other cell types, such as podocytes[21]. It is difficult to distinguish renal stem/progenitor cells from dedifferentiated renal epithelial cells. Identification of specific markers that are exclusively expressed in stem/progenitor cells, but not in dedifferentiated renal epithelial cells, will be required to resolve this issue.

Mesenchymal stem cells

The MSCs reside in various organs, such as bone marrow, subcutaneous adipose tissue, and skeletal muscles. Bone marrow mesenchymal stem cells (BMMSCs) and adipose tissue-derived MSCs (ADSCs) are particularly interesting, because a large amount of MSCs can be collected with relatively less invasive procedures.

BMMSCs: Many studies have demonstrated the efficacy of BMMSCs in the treatment of kidney disease using animal models of AKI[22-26], podocyte injury[27], glomerulonephropathy[28-30], a remnant kidney[31], and kidney transplantation[32] (Table 2). Although early studies indicated BMMSCs could be differentiated into renal epithelial cells[22,23] and mesangial cells[30], recent evidence suggests that the differentiation capacity of BMMSCs is limited. Thus, BMMSCs do not appear to differentiate into renal parenchymal cells *in vivo*[33]. The beneficial effects of BMMSCs on renal function seem to be largely mediated by paracrine factors produced by BMMSCs that have anti-apoptotic, proangiogenic, and/or immune modulatory effects[34-36]. The transdifferentiation of BMMSCs observed in early reports may reflect cell fusion. If BMMSCs fuse with resident cells in the kidney, they acquire a phenotype of resident cells. Thus, it appears as if BMMSCs were differentiated into resident cells. Indeed, several reports have demonstrated that BMMSCs are capable of fusing with other cell types[37,38]. A phase Ⅰ clinical study evaluated the safety and efficacy of allogenic BMMSC administration for the prevention of AKI after open-heart surgery[39]. This study enrolled 16 patients who required on-pump cardiac surgery and who were at a high risk of postoperative AKI due to underlying chronic kidney disease (CKD), advanced age, diabetes mellitus, and congestive heart failure. Allogenic BMMSCs were injected into the suprarenal aorta after surgery. The primary objective was the safety of BMMSC administration. The secondary objective was the efficacy of this treatment compared with well-matched historical controls. This treatment appeared to be both safe and effective as no adverse events related to the procedure were reported, and renal function was well preserved post-operatively, with no patients requiring hemodialysis after surgery, whereas 20% of the controls developed AKI. This is the only clinical trial published so far in which ASCs were used to treat kidney disease.

ADSCs: ADSCs are another type of MSCs residing

Table 3 Effects of adipose tissue-derived mesenchymal stem cells on renal tissue repair

Origin of stem cells	Experimental model	Effects	References
Rat	I/R injury	Recovery of renal function Reduction in oxidative stress	Chen *et al*[40]
Human	Cisplatin-induced AKI	Recovery of renal function Conditioned medium was effective	Kim *et al*[41]
Human	Folic acid-induced AKI	Recovery of renal function HGF and VEGF production	Katsuno *et al*[42]
Rat	Anti-GBM disease	Reduced renal injury and proteinuria Conversion of macrophages to immunoregulatory cells	Furuhashi *et al*[43]
Swine	Renal artery stenosis	Recovery of renal function Improved angiogenesis Increased production of VEGF and bFGF	Eirin *et al*[44]
Swine	Renal artery stenosis	Recovery of renal function Improved angiogenesis Decreased oxidative stress	Zhu *et al*[45]
Swine	Renal artery stenosis	Recovery of renal function Improved angiogenesis Increased production of VEGF	Ebrahimi *et al*[46]
Mouse	Renal fibrosis (unilateral clamping of the renal pedicle)	Recovery of renal function Reduced fibrosis Decreased expression of IL6 and TNF-α	Donizetti-Oliveira *et al*[47]

I/R: Ischemia reperfusion; AKI: Acute kidney injury; GBM: Glomerular basement membrane; HGF: Hepatocyte growth factor; VEGF: Vascular endothelial growth factor; bFGF: Basic fibroblast growth factor; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-α.

in subcutaneous adipose tissues. Because the subcutaneous adipose tissues are abundant and can be easily harvested using liposuction, ADSCs are promising stem cells for clinical use. The efficacy of ADSC administration in the treatment of kidney disease has been demonstrated in animal models of AKI[40-42], glomerulonephropathy[43], renal artery stenosis[44-46], and progressive renal fibrosis[47] (Table 3). ADSCs also seem to recover renal function largely *via* paracrine effects[41,42].

EPCs: EPCs were originally isolated from human peripheral blood using CD34 as a marker for positive selection[48]. The CD34+ mononuclear blood cells obtained the characteristics of vascular endothelial cells (VECs) when cultured on fibronectin-coated dishes. EPCs were reportedly incorporated in ischemic tissues *in vivo* and expressed markers for VECs such as CD31 when introduced into the circulation using a hindlimb ischemia model. The efficacy of EPC administration in the recovery of renal function was reported in animal models of AKI[49] and renal artery stenosis[50,51]. Interestingly, the function of EPCs was deteriorated in CKD patients[52], suggesting that the autologous transplantation of EPCs may not be suitable for the treatment of CKD.

Umbilical cord blood-derived MSCs

Umbilical cord blood (UCB) contains MSCs, and the efficacy of UCB administration in the restoration of renal function has been reported in animal AKI models[53,54]. Morigi *et al*[53] injected human UCB-derived MSCs to immunodeficient mice with cisplatin-induced acute tubular injury. They demonstrated that these cells ameliorated tubular injury, resulting in the recovery of renal function. They also cocultured UCB-derived MSCs with cisplatin-treated proximal tubular cells (HK-2 cells) and demonstrated that the expression of HGF was particularly induced and that of interleukin 1-β and tumor necrosis factor-α was significantly decreased in the coculture system. These findings suggested that the modulation of paracrine factors in the kidney was implicated in the UCB-induced recovery of renal function. Panepucci *et al*[55]. compared the gene expression profile of UCB-derived MSCs and BMMSCs. Although both MSCs expressed similar sets of genes, BMMSCs predominantly expressed a set of genes related to antimicrobial activity and osteogenesis, whereas UCB-derived MSCs predominantly expressed genes related to matrix remodeling and angiogenesis, suggesting that UCB-derived MSCs and BMMSCs may have distinct activities *in vivo*.

Amniotic fluid stem cells

Human amniotic fluid contains stem cells derived from embryos, and thus, is a promising source of stem cells. The efficacy of human amniotic fluid stem cells (HAFSCs) has been demonstrated in animal models of AKI[56,57]. Houser *et al*[56] compared the characteristics of HAFSCs with that of BMMSCs. They found that compared with BMMSCs, HAFSCs had a more potent anti-apoptotic activity against renal tubular cells but lesser stimulatory activity for the proliferation of renal tubular cells. They also demonstrated that HAFSCs and BMMSCs expressed distinct sets of paracrine factors, suggesting that HAFSCs and BMMSCs may have distinct activities *in vivo*.

Direct/indirect reprogramming of adult somatic cells

Another strategy for kidney regeneration is to create

pluripotent stem cells and progenitor cells, whose destination is limited to one or several cell lineages, or terminally differentiated renal parenchymal cells from adult somatic cells *via* direct/indirect reprogramming. Indirect reprogramming is a strategy in which adult somatic cells are induced to dedifferentiate into pluripotent stem cells and re-differentiate into specific cell types. Direct reprogramming involves a strategy in which adult somatic cells are induced to differentiate directly into another cell type. Since the discovery of induced pluripotent stem (iPS) cells[58,59], it is not difficult to prepare iPS cells from various adult somatic cells. Indeed, several reports have demonstrated a successful preparation of nephrogenic intermediate mesoderm, from which the MM and the UB derive, using iPS cells[60-62]. However, several hurdles remain to be overcome before iPS cells can be used practically to regenerate the kidney. First, the efficiency of iPS cell preparation from adult somatic cells is still low. Second, it is still challenging to prepare kidney stem/progenitor cells that exist in the CM from iPS cells. Third, even if kidney stem/progenitor cells are successfully created from iPS cells, it is difficult to continue to culture and expand those stem/progenitor cells while maintaining their unique properties. The reason for this limitation is that the niche for kidney stem/progenitor cells has not been clearly understood. Therefore, further studies will be required to use indirect reprogramming for kidney regeneration. Recently, several reports have demonstrated that a direct reprogramming method was useful for kidney regeneration. Hendry *et al*[63] introduced 6 transcription factors into a human adult renal proximal tubular cell line. These cells were localized in *Six2*+ and Wilm's tumor 1+ compartment in an *ex vivo* organoid culture assay. These findings suggest that these cells obtained properties similar to kidney stem/progenitor cells. Papadimou *et al*[64] incubated permeabilized human BMMSCs with the extracts of human proximal tubular epithelial cells and obtained a cell population similar to proximal renal tubular cells. These cells were integrated in tubular structures in an *ex vivo* organoid culture assay. Furthermore, these cells were engrafted in renal tubules when administered to a cisplatin-induced AKI model. Therefore, direct reprogramming seems to be a promising strategy for kidney regeneration. It has been reported that iPS cells derived from various cell types are not identical in their differentiation capacity[65-67], probably because iPS cells maintain epigenetic memory of their parental cells. Thus, it may be better to use renal parenchymal cells for reprogramming than cells derived from tissues other than the kidney.

Possible roles of extracellular vesicles released from MSCs

MSCs not only secrete bioactive molecules directly into the circulation but also release extracellular vesicles (EVs)[68], such as exosomes that contain proteins, mRNA, and microRNA[69,70]. Several reports have demonstrated that EV administration restored the kidney function in animal models of AKI[26,71-73]. Bruno *et al*[71] isolated EVs from supernatants of human BMMSCs and examined their effects on the proliferation and apoptosis in renal epithelial cells. EVs were incorporated in renal epithelial cells *in vitro* and the incorporation depended on CD44 and β1-interin. EVs stimulated the proliferation and inhibited apoptosis of renal epithelial cells. These effects were diminished when EVs were treated with RNase prior to administration. Furthermore, the authors administered EVs to immunodeficient mice with glycerol-induced acute tubular injury and demonstrated that EV administration restored renal function. Moreover, these beneficial effects were diminished when EVs were pretreated with RNase. Tomasoni *et al*[74] isolated EVs from supernatants of human BMMSCs and demonstrated that EVs contained mRNA for the insulin-like growth factor-1 receptor (IGF1R). When cisplatin-treated renal epithelial cells were incubated with EVs, proliferative capacity of renal epithelial cells increased significantly; however, the stimulatory effect was diminished when the expression of IGF1R mRNA in BMMSCs was suppressed using small interfering RNA to IGF1R prior to EV harvest. Zhou *et al*[75] administered EVs harvested from human UCB-derived MSCs to a rat model of cisplatin-induced AKI. EV administration significantly restored renal function and morphology. Therefore, EVs seem to contain various bioactive molecules that can be used for the treatment of kidney injury.

FUTURE DIRECTIONS

It seems that there are two major directions to improve the quality of stem cell therapy for kidney diseases. One is to analyze bioactive molecules released from stem cells in more details. If an ideal combination of bioactive proteins, mRNA, and/or microRNA is elucidated, stem cells *per se* will not be necessary in the future. Although BMMSCs have been used in the clinical setting to treat patients with cardiovascular disease[76-80], concern about tumorigenesis still remains[81]. Furthermore, BMMSCs isolated under uremic conditions have less capacity for the proliferation, survival, and secretion of paracrine factors compared with those isolated from normal controls[82-84]. Patients who need stem cell therapy are probably not a suitable source of high-quality stem cells, indicating that autologous stem cell transplantation may not be effective in these patients. Therefore, cell-free therapy seems attractive. Another alternative is to explore strategies to directly and/or indirectly reprogram adult somatic cells into kidney stem/progenitor cells. If efficient and safe methods to induce direct/indirect reprogramming are explored, bona fide kidney regeneration rather than kidney repair using remnant renal parenchymal cells will be possible in the future.

CONCLUSION

Although intensive studies have been performed to isolate kidney stem/progenitor cells from the adult

kidney, it is debated whether kidney stem/progenitor cells actually exist in the adult kidney. There are no specific markers and/or assays to discriminate kidney stem/progenitor cells from dedifferentiated renal epithelial cells. MSCs are effective to repair the kidney in various animal models; however, their beneficial effects can be largely attributed to paracrine factors that are secreted from MSCs. In addition, MSCs release EVs that contain mRNA and microRNA as well as proteins. These EV-derived molecules may also play beneficial roles in the repair of the kidney. It will be necessary to elucidate ideal combinations of the molecules released from MSCs to establish a strategy for the maximal stimulation of kidney repair without using stem cells. It will also be necessary to explore strategies to directly and/or indirectly reprogram somatic cells to kidney stem/progenitor cells to regenerate the kidney.

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REVIEW

Baroreflex dysfunction in chronic kidney disease

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Abstract

Chronic kidney disease (CKD) patients have high cardiovascular mortality and morbidity. The presence of traditional and CKD related risk factors results in exaggerated vascular calcification in these patients. Vascular calcification is associated with reduced large arterial compliance and thus impaired baroreflex sensitivity (BRS) resulting in augmented blood pressure (BP) variability and hampered BP regulation. Baroreflex plays a vital role in short term regulation of BP. This review discusses the normal baroreflex physiology, methods to assess baroreflex function, its determinants along with the prognostic significance of assessing BRS in CKD patients, available literature on BRS in CKD patients and the probable patho-physiology of baroreflex dysfunction in CKD.

Key words: Large arterial compliance; Chronic kidney disease; Vascular calcification; Baroreflex sensitivity; Blood pressure variability

Core tip: Cardiovascular dysfunction is an important complication and risk factor of mortality and morbidity in chronic kidney disease (CKD). Baroreflex is a functional integrator of cardiovascular homeostasis. Derangement in baroreflex function is not only a manifestation of cardiovascular pathogenesis in general and in CKD but also contribute to ongoing etio-pathogenesis. The present review discusses the physiology and dysfunction in CKD in light of the available literature.

Kaur M, Chandran DS, Jaryal AK, Bhowmik D, Agarwal SK, Deepak KK. Baroreflex dysfunction in chronic kidney disease. *World J Nephrol* 2016; 5(1): 53-65 Available from: URL: http://www.wjgnet.com/2220-6124/full/v5/i1/53.htm DOI: http://dx.doi.org/10.5527/wjn.v5.i1.53

INTRODUCTION

Most common etiology of mortality and morbidity in chronic kidney disease (CKD) patients are cardiovascular events, rather than uremia itself. Interestingly, CKD is now recognized as an independent risk factor for cardiovascular disease[1,2]. Practice guidelines from the National Kidney Foundation 2002 recommend that CKD be considered a coronary artery disease risk equivalent[3].

Cardiovascular abnormalities in CKD includes both cardiomyopathy (left ventricular hypertrophy) and vasculopathy (arteriosclerosis and atherosclerosis) - which ultimately culminates to ischemic heart disease and cardiac failure[4,5] (Figure 1).

Clinical presentation of cardiovascular disease in CKD includes hypertension, left ventricular hypertrophy, congestive heart failure, myocardial infarction and sudden death. Moreover in end stage renal disease (ERSD) patients, the prevalence of left ventricular hypertrophy and coronary artery disease are 75% and 40%, respectively. Death from cardiac causes is 10-20 times more common in patients with ESRD than in age matched segments of the general population and amounts for almost 30% to 50% of all death[4,6-8].

Mechanism of cardiovascular dysfunction

CKD is associated with both traditional and CKD related risk factors. Furthermore, the presence of added CKD related (non-traditional) risk factors in this population accounts for the exorbitant cardiovascular risk in these patients[9-14] as listed in Table 1 (Sarnak *et al*[15]).

Cumulative effect of ensemble of these risk factors results ultimately to vascular calcification in CKD patients[9,10,16]. Central to the pathogenesis of cardiovascular dysfunction is vascular calcification[16-19]. Reviews are available discussing the mechanism of vascular calcification in CKD patients[16,20-29]. Vascular calcification results in stiffer arteries with reduced compliance[18,28]. Reduction in compliance of central arteries not only results in higher afterload and diminished perfusion of heart (London *et al*[30]) but also impaired baroreflex sensitivity (BRS)[31-34].

Baroreflex is a major regulatory mechanism for buffering short-term blood pressure (BP) fluctuations by modulating the heart rate and vascular tone. Baroreflex loop functioning is an important indicator of integrity of cardiovascular homeostatic regulation. Impaired baroreflex function results in loss of dampening of BP fluctuations and thus higher BP variabilities[34,35]. Higher blood pressure variability (BPV) has been associated with end-organ damage[36-38]. Previously a study by Kaur *et al*[34] proposed a model for showing the improvement in baroreflex function after renal transplantation (RT) in ESRD patients discussing the relationship between BRS, arterial stiffness and BPV and found that RT results in improvement in arterial stiffness followed by normalization in BRS and reduction in BPV. This highlights the significance of baroreflex function in CKD. The purpose of this review is to consolidate the published evidence on baroreflex physiology, methods of assessment, its determinants and dysfunction in CKD patients.

PHYSIOLOGY OF BARORECEPTOR REFLEX

Baroreceptor reflex (baroreflex) plays a significant role in the short term regulation of arterial BP. Pioneering works on animal models by Hering, Korner, Cowley, Guyton and others have clearly implicated its role in buffering arterial BP fluctuations induced by internal and external perturbations[39-41]. Evidence is currently accumulating in support of the hypothesized role of baroreceptors in long term regulation of arterial BP as well[42].

Baroreceptors are stretch sensitive receptors located in the high pressure (high pressure baroreceptors) as well as low pressure (low pressure baroreceptors) areas of the circulatory system. High pressure arterial baroreceptors located in the Carotid sinus and Aortic arch (Sinoaortic baroreceptors) are considered to play a dominant role in the moment to moment regulation of arterial BP. Considering this fact, alterations in arterial baroreflex mechanisms have been implicated in clinical disorders characterized by abnormal fluctuations in BP imposed commonly by postural variations. This section of the review would cite and discuss literature relevant to understand the physiology of arterial baroreflex and the methods of assessment of arterial BRS in human subjects and patients.

Baroreflex arc

Sino-aortic baroreceptors provide the cardiovascular regulatory centres in the brainstem with a continuous stream of information on the beat to beat fluctuations in BP. These stretch sensitive receptors are encapsulated or free nerve endings located in the tunica adventitia of carotid sinus and aortic arch that respond to the changes in dimensions of the arterial wall produced by fluctuations in transmuralpressure. Afferent information from the receptors are relayed to brainstem nuclei through glossopharyngeal (afferents from carotid sinus) and vagus nerves (afferents from aortic arch) which act as the centre of the baroreflex arc (Figure 2). Baroreceptor inputs to brain stem primarily reach the nucleus tractus solitarius (NTS) located in the dorsal medulla which has intricate connections with the cardioinhibitory and vasomotor centers located in the caudal and rostral ventrolateral medulla (RVLM) and the nucleus ambiguus of vagus. RVLM projects to preganglionic sympathetic neurons located in the intermediolateral gray column of thoracic and lumbar spinal segments. Axons of the neurons in nucleus ambiguus project as pre-ganglionic parasympathetic supply to the heart. Figure 2 depicts the neuronal circuitry of the baroreflex arc.

Baroreflex activation and effector responses

NTS continuously receives a tonic input from sino-aortic baroreceptor afferents which discharge in phase with the

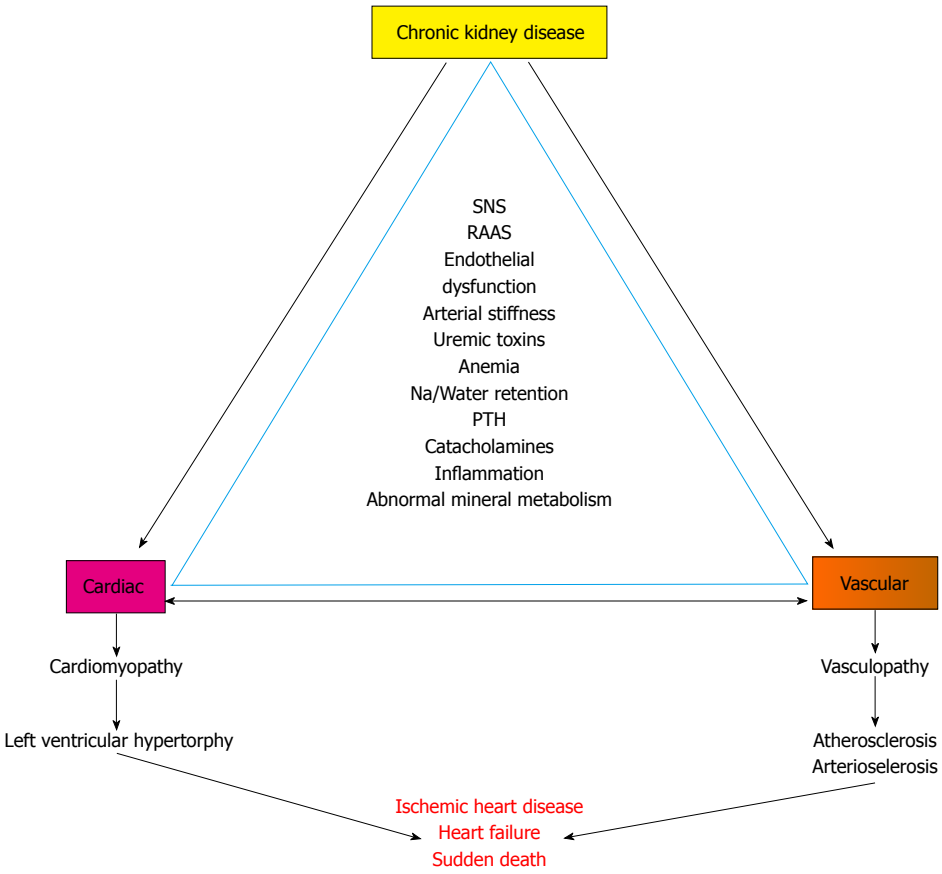

Figure 1 Cardiovascular abnormalities in chronic kidney disease. Depicts the association of chronic kidney disease related risk factors and cardiac and vascular abnormalities and outcomes in chronic kidney disease. SNS: Sympathetic nervous system; RAAS: Renin angiotensin aldosterone system; Na: Sodium; PTH: Parathyroid hormone.

arterial pressure waveform. Within the operating range, the frequency of discharge in baroreceptor afferents responds to changes in both the magnitude and slope of arterial pressure waveform. A rise in systemic mean arterial and/or pulse pressure would lead to an increased discharge in the baroreceptor afferents phase-locked with the arterial pressure waveform. Increase in baroreceptor input to NTS initiates reciprocal changes in the efferent vago-sympathetic discharge leading to increased firing of cardioinhibitory vagal neurons innervating sino-atrial node and decreased firing of sympathetic neurons controlling heart and peripheral blood vessels. This would produce a decrease in heart rate mainly through the vagal limb and a decrease in cardiac contractility, peripheral vascular resistance and venous return through the sympathetic limb. All these changes will ultimately bring the BP down, close to its set point thereby instituting negative feedback control to establish circulatory homeostasis. Thus, baroreflex arc may be considered to operate through two physiologically antagonistic efferent pathways comprising of vagal and sympathetic fibres innervating heart and peripheral blood vessels. The vagal limb is quick to act with latencies as low as 200 ms to 600 ms in comparison to the sympathetic limb which takes more than 2 s to 3 s to produce any noticeable change in the cardiac contractility or peripheral resistance. This discrepancy is largely attributed to the obvious differences in cholinergic and adrenergic signal transduction mechanisms at the target cells.

DETERMINATION OF BRS - METHODOLOGICAL CONSIDERATIONS

Quantification of BRS has largely been part of experimental laboratory work in animal models and human subjects until recently when clinical investigations started revealing impaired BRS as a pathophysiological entity in cardiovascular disorders[43-45]. Moreover, BRS estimation has been attributed immense prognostic value in predicting cardiac mortality in the large multicentric autonomic tone and reflexes after myocardial infarction study[46]. Similar observations have also been reported in a group of patients with mild to moderate heart failure, signifying the role of BRS as a prognostic indicator in the risk stratification of patients[47].

From a physiological control system perspective, baroreceptor reflex is considered to operate in closed loop with open loop characteristics, *i.e.*, changes in BP elicit appropriate heart rate responses through open loop negative feedback mechanisms which tend to buffer the initiating change in BP through a feedforward influence of heart rate on BP that closes the loop[48,49]. Majority of

Table 1 Cardiovascular risk factors in chronic kidney disease

Traditional risk factors	Non-traditional factors
Sympathetic hyperactivity	Albuminuria
Hyperhomocysteinemia	Inflammation
Hypertension	Oxidative stress
High LDL cholesterol	Anemia
Low HDL cholesterol	Abnormal calcium/phosphate metabolism
Diabetes	Extracellular fluid volume overload
Smoking	Electrolyte imbalance
Physical inactivity	Malnutrition
Menopause	Sleep disturbances
Family history of CVD	Endothelial dysfunction

LDL: Low density lipoprotein; HDL: High density lipoprotein; CVD: Cardiovascular disease.

the BRS assessment protocols ignore the feedforward influence considering it as inconsequential and compute BRS as the feedback gain of the open loop[50,51].

Methodologically, BRS assessment strategies can be broadly categorized into (1) those based on artificially imposed changes in arterial BP or carotid sinus pressure including pharmacological methods, Valsalva maneuver and neck chamber techniques; (2) those based on analysis of spontaneous oscillations in BP and heart rate.

Methods based on artificially imposed changes in arterial BP or carotid sinus pressure

These methods use physiological maneuvers or pharmacological agents to impose changes in BP. The resulting baroreflex mediated changes in heart intervals are simultaneously acquired along with BP signal and subjected to appropriate analysis to derive various estimates of BRS.

Pharmacological method

Pharmacological method, also termed as the "Oxford technique" involves intravenous administration of graded bolus doses of a suitable vasoconstrictor agent to produce rise in BP that would lead to baroreflex induced bradycardia[51-53]. Phenylephrine, a pure alpha adrenoceptor agonist is the commonly preferred vasoconstrictor agent as it is considered to have minimal extravascular effects. Many investigators prefer to administer in addition, a vasodilator agent (sodium nitroprusside infusion or amyl nitrite by inhalation) to induce fall in BP to precipitate baroreflex mediated increase in heart rate to capture responses on either side of the setpoint. Beat to beat BP and ECG signals are simultaneously recorded during the periods when BP rises above and below the resting baseline values under the influence of the vasoactive agents. Consecutive systolic BP values are plotted against the simultaneously recorded RR intervals or pulse intervals with one beat delay to fit the linear regression line between the two variables. BRS is computed as the slope of this line and expressed in ms/mm of Hg. Despite being invasive, pharmacological method is the commonly employed method to estimate BRS for risk stratification of patients owing to its repeatability and accuracy.

Valsalva's maneuver

Valsalva's maneuver is one of the earliest known physiological maneuvers used to study the baroreflex function in humans. Performance of the maneuver involves forced expiration against a closed or partly open glottis to raise the intrathoracic and intraabdominal pressures with secondary hemodynamic effects. The maneuver physiologically imposes fall in BP due to decreased venous return during phase Ⅱ and rise in BP during phases Ⅳ due to uninterrupted venous return to an already stimulated heart. The corresponding baroreflex mediated RR interval changes are acquired simultaneously with the beat to beat BP values. A linear regression analysis is commonly performed between consecutive systolic BP values and corresponding RR intervals with one beat delay during phase Ⅳ to derive BRS (also known as cardiovagal gain) as the slope of the fitted line[51,54]. Estimation of BRS by Valsalva's manoeuvre has been reported to be non-selective for arterial baroreflex as it also engages other low pressure baroreceptors into action[55].

Neck chamber technique

The neck chamber technique[56,57] produces activation or deactivation of carotid baroreceptors through a graded application of negative or positive pneumatic pressure around the neck region. Negative neck pressure increases the carotid sinus transmural pressure leading to increased stretching of its wall and afferent baroreceptor firing. This would induce a fall in systemic arterial pressure consequent to baroreflex mediated changes in heart rate, cardiac contractility, peripheral resistance and venous return. BRS is computed by linear regression analysis using the transmural carotid sinus pressure and RR interval data acquired during the phases of manipulation. Neck chamber technique is the only method which selectively estimates the carotid BRS. However, it has been sparingly used in clinical investigations with most of the available literature citing its use relate to studies conducted in association with experimental laboratory work.

Methods based on analysis of spontaneous oscillations in BP and heart intervals

Since 1980s, with the invention and widespread use of non-invasive beat to beat BP monitors based on the volume clamp principle of "Penaz", there has been tremendous progress in the development of purely non-invasive methods of BRS assessment. This newer generation of techniques employs computer based analysis of spontaneous oscillations in the BP coupled with reflex changes in heart rate to derive estimates of BRS. The spontaneous BRS estimation methods can be broadly categorised into time domain and frequency domain methods.

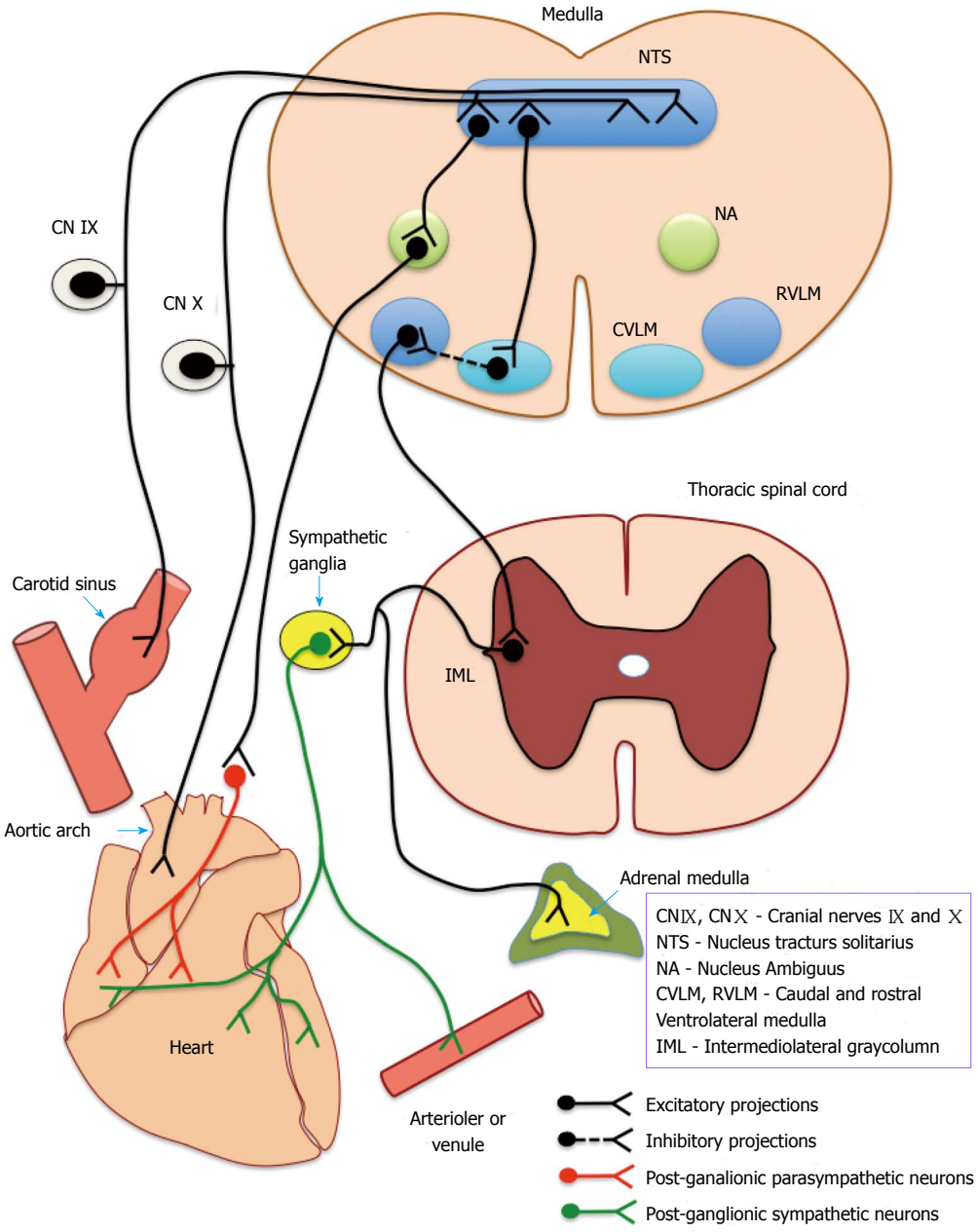

Figure 2 Neuronal circuitry of the baroreflex arc. Depicts the complete baroreflex arc - beginning from the baroreceptors (located in carotid sinus and aortic arch), afferents (Ⅸ and Ⅹ cranial nerve) ascend to medullary centres and send efferents (sympathetic and parasympathetic) to end organs (heart and vasculature). CN Ⅸ and CN Ⅹ: Cranial nerve Ⅸ and Ⅹ; NTS: Nucleus tractus solitarius; NA: Nucleus ambiguous; CVLM: Caudal ventrolateral medulla; RVLM: Rostral ventrolateral medulla; IML: Intermediolateral gray column.

Time domain methods

Time domain methods analyse the time series data of beat to beat BP and heart intervals to estimate BRS. In the "sequence method" proposed by Parati *et al*[50,51,58], the algorithm automatically searches and identifies "sequences" in which BP shows a continuous increase (or decrease) for at least three consecutive beats that is accompanied by lengthening (or shortening) of consecutive RR intervals with zero to two beats delay. Sequence method considers the associated RR interval changes as baroreflex mediated response to the spontaneously emerging ascending or descending pressure ramps. A linear regression analysis between the BP and RR interval variables will derive the slope of the best fit line as estimated BRS.

Frequency domain methods

The spectral or frequency domain methods are based on the principle that, spontaneous oscillations in BP centered around a particular frequency will lead to baroreflex mediated oscillations in heart interval in the same frequency. The ratio of the powers of the oscillations estimated by autoregressive or other methods in a particular frequency band or the modulus of the transfer function relating BP with heart interval oscillations are computed as the BRS estimates[50,51]. Oscillations in

two frequency bands are usually being taken for the computations; a low frequency band centered around 0.1 Hz (ranging from 0.04 to 0.15 Hz) and a high frequency band of respiratory origin ranging from 0.15 to 0.4 Hz. One of the commonly used spectral methods estimates BRS as the root-squared ratio between heart interval and systolic pressure powers calculated in the LF band (α LF) or in the HF band (α HF). These spectral indices are considered to be valid when the linear correlation (coherence) between BP and heart rate oscillations in the specified frequency bands are sufficiently high. The transfer function method proposed by Robbe *et al*[59], computes BRS as the modulus or gain of the transfer function between variations in BP and heart interval in a specified frequency band. Transfer function is usually computed for both low frequency and high frequency bands deriving two different estimates of BRS named H_{LF} and H_{HF} respectively. Other spectral methods for estimating BRS include describing the spontaneous oscillations in BP and heart intervals using mathematical models and deriving BRS using the model coefficients.

Choice of the appropriate method of BRS assessment in clinical setting

Despite being invasive, pharmacological method is the most preferred technique for BRS estimation by most clinical investigators owing to its repeatability across different populations of patients. With the advent of non-invasive beat to beat BP monitors, impetus on the usage of spontaneous methods as replacement for the invasive pharmacological method has been steadily growing. Sequence method is considered to be the physiological replica of pharmacological method since both the techniques analyse heart interval responses to ascending or descending pressure ramps originating spontaneously or in response to vasoactive agents. However, many investigators believe spectral indices to give better and reliable estimates of BRS comparable to that obtained by invasive pharmacological methods[59-61]. Choice of the most appropriate spontaneous method of BRS estimation is dependent on experimental factors and stationarity of the BP and heart interval signals. Reliable estimation of BRS by spectral methods is guaranteed only if the blood pressure and heart rate signals are stationary during the selected window of analysis. Sequence method is preferred over spectral indices if the stationarity of the signals cannot be ensured[50,51]. Majority of the initial reports on baroreflex functions in CKD patients have employed pharmacological method[62-65] to estimate BRS while a few have also used Valsalva maneuver[66]. Spontaneous sequence and spectral methods have also been utilized in the studies conducted in the recent past[34,67].

DETERMINANTS OF BRS

Factors determining BRS can broadly be categorized as demographic and physiological, as reported by multiple studies conducted in healthy subjects and patients using both invasive and non-invasive methods. Age, gender, systolic and diastolic BP, resting heart rate and body mass index have been reported as the major determinants of BRS[68,69]. The relationship between age and BRS was observed to be physiologically linked through age related changes in carotid distensibility[70-73]. Age related decline in carotid distensibility tends to minimise the diameter changes associated with arterial pressure fluctuations, thereby reducing the transduction abilities of sino-aortic baroreceptors. This has been corroborated by direct estimation of carotid distensibility coefficients and its correlation with BRS as quantified by pharmacological method in healthy human subjects[74]. Central arterial stiffness as measured by aortic pulse wave velocity has been reported to be an independent predictor of BRS by the Rotterdam cohort study conducted in 2083 elderly subjects[75]. Reduction in arterial distensibility associated with stiffening of the central arteries and a consequent fall in BRS is one of the possible mechanisms implicated in baroreflex dysfunction in CKD patients.

PROGNOSTIC SIGNIFICANCE OF BAROREFLEX ASSESSMENT IN CKD

BRS is emerging as a cardinal prognostic risk factor in CKD patients. Johansson *et al*[76] studied BRS in hypertensive CKD patients and then followed them up prospectively for 41 +/- 15 mo and found that 69 patients died during the follow-up. Cardiovascular diseases and uremia resulted in the majority of deaths (60% and 20%, respectively), while sudden cardiac death occurred in 15 patients. Reduced BRS was found to be an independent predictor of sudden cardiac death (RR = 0.29; 95%CI: 0.09-0.86 for an increase of one standard deviation in BRS, P = 0.022). The authors concluded that BRS may convey important prognostic information that will have clinical implications for patients with CKD.

Reduced BRS is also associated with hemodialysis related hypotension, which results in significant mortality in hemodialysis patients as they are unable to counteract dialysis induced volume depletion[66,77]. Chesterton *et al*[31], reviewed the importance of assessment of BRS in CKD patients, especially its relevance in prediction of vasomotor instability during dialysis. The authors inferred from literature that there are demonstrable pathological alterations in CKD, contributing to structural and functional changes in the cardiovascular system that may result in both haemodynamic instability and cardiovascular mortality. Understanding the associations between conventional markers of haemodynamic instability and BRS (as a measure of autonomic function) will allow early and better risk stratification, prevention and managementin CKD patients.

EVIDENCE OF IMPAIRED BRS IN CKD

Baroreceptor reflex control, as studied by BRS is reduced in CKD patients and worsenswith the disease severity (Table 2). Studies have also compared BRS of patients on different treatment modality of CKD - hemodialysis, peritoneal dialysis and RT with inconsistent results. Although the literature on BRS assessment in CKD is scarce, but most existing studies suggest that dialysis fails to improve BRS in CKD patients while renal transplant undoubtedly improves it. Few studies have also examined the correlation of BRS with vascular compliance and autonomic parameters in-order to understand the pathophysiology of baroreflex dysfunction in CKD patients. Although this still remains to be studied in further details.

CONCEPTUAL MODEL EXPLAINING THE REDUCED BRS IN CKD

We have previously seen in the earlier section (Figure 2) the complete baroreflex arc. Conceptually a defect anywhere in this loop could result in impaired BRS in CKD.

Till now different schools of thoughts have been categorized to summarize the defect in baroreflex function in CKD: (1) Vascular *vs* Neural debate; (2) Structural *vs* functional mechanisms.

As a matter of fact, none of these contemplations are full-proof and mutually exclusive. There exists a grey area of overlap of these factors resulting in baroreflex dysfunction.

In the next section, we will discuss the limited evidence available to possibly speculate the pathophysiology of reduction in BRS in CKD patients.

PATHOPHYSIOLOGY OF BAROREFLEX DYSFUNCTION IN CKD

Vascular vs neural

The baroreflex arc is integral to the short-term regulation of BP and is under autonomic regulation. A change in BP results in an alteration in transmural stretch within the baro-sensitive central arteries. This causes activation of the baroreceptors (level 1 in Figure 3) located within the adventitia of arterial wall. Modified firing from these receptors is transmitted *via* the afferent nerves (level 2 in Figure 3) to the central autonomic centre (level 3 in Figure 3). Sympathetic and parasympathetic systems (level 4 in Figure 3) influencing vessels and heart (level 5 in Figure 3) constitute the efferent response. Thus, a change in BP results in a corresponding change in the RR interval and vessel tone, restoring BP to normal limits.

BRS is well recognized as a composite marker of the overall integrity of the baroreflex arc[31]. BRS is therefore determined by the mechanical properties of the arterial wall which constitutes the vascular component, and the parasympathetic and sympathetic nervous system forming the neural component.

Chesterton *et al*[31] found that BRS is impaired in CKD which explains the development of intra-dialytic hypotension (IDH) in these patients. IDH is associated with increased mortality in hemodilaysis (HD) patients. Additionally, they investigated the link between vascular calcification (measure of arterial structure), arterial stiffness (measure of arterial function) and BRS in chronic HD patients and concluded that there is a positive association between vascular calcification and BRS. Thus the impaired BRS observed in CKD patients could be due to the excessive vascular calcification observed in them.

In concordance Kaur *et al*[34] studied the reversibility of arterial stiffness indices along with BRS before, at 3 mo and 6 mo after RT in-order to understand the temporal connection between these parameters. They reported the normalization of BRS in ESRD patients by 6 mo which followed the early improvement in arterial stiffness.

On similar lines, Boutouyrie *et al*[83] also theoretically categorized the baroreflex loop into vascular compartment which includes the wall stretch component (receptor level) and neural comprising of afferent, centre and efferent arc of baroreflex. Notably, baroreceptors embedded in the adventitia of central arteries are sensitive only to vessel wall stretch and not directly to intravascular pressure. Pressure changes inside the vascular lumen need to get translated as vessel wall stretch to get sensed by baroreceptors. This pressure to stretch conversion is dependent on arterial compliance and thus, stiffness of large arteries become a crucial determinant of the vascular component of baroreflex[74,84]. CKD is associated with both vascular remodelling and autonomic dysfunction. The authors commented on a previous study[34] and discussed that questions still remain regarding how transplantation improves baroreflex - is it through amelioration of arterial properties or neural components or/and a relative contribution of both.

This puzzle remains unresolved till date. Most available data is suggestive of a probable defect at level 1 that is the sensing by baroreceptors itself. Although there exists data regarding dysfunction at other sites also in human and animal studies - level 2 - afferents[85,86], level 3 - centre[86,87], level 4 - efferents[85,88,89] and level 5 - end organ[90-92] in CKD.

By studying in detail the large artery and neural parts components of baroreflex arc, studies in future may help in understanding this concept further.

Structural vs functional modulation of the arterial baroreflex

Large artery structural changes are considered to be the predominant mechanism responsible for decreased BRS[74]. There is an emerging concept of the role of "functional mechanisms" responsible for altered baroreflex function which could be either at the level of peripheral sensory endings and/or at the central nervous system.

Table 2 Baroreflex sensitivity in chronic kidney disease

Ref.	Number of patients Study design	Method of BRS assessment	Results
Pickering *et al*[65]	32 patients on HD serially studied	Intra-venous bolus of phenylephrine	BRS was found to be low HD improved reflex sensitivity over the long term, but did not have any consistent immediate effect
Lazarus *et al*[64]	13 patients on HD and 5 controls Cross- sectional	Intra-venous angiotensin and inhaled amyl nitrite	BRS lower in patients than controls for both pressor and depressor stimuli
Tomiyama *et al*[78]	22 non-dialysed patients and controls	Intra-venous bolus of phenylephrine and inhaled amyl nitrite	Lower BRS in patients as compared to controls
Agarwal *et al*[62]	Cross- sectional 25 non-dialyzed patients and 8 controls 8 patients reassessed after 6.6 +/- 1.0 wk of hemodialysis 12 patients were restudied 24 +/- 4.0 wk after renal transplantation	Intra-venous bolus of phenylephrine	Lower BRS in patients 8 patients restudied after HD, BRS lower in hypotension-prone *vs* normotensive group 12 patients restudied after RT, BRS improved
Gerhardt *et al*[67]	20 patients of HD, RT and controls each Cross-sectional	Sequence analysis	Reduced BRS in CKD *vs* Controls Similar BRS in RT and controls
Gao *et al*[79]	17 ESRD patients and 29 controls Cross-sectional	Sequence analysis	BRS was 62% lower in ESRD than controls
Johansson *et al*[80]	216 hypertensive CKD patients with 43 age-matched controls	Spontaneous method	BRS was reduced by 51% in CKD patients as compared with controls Greater reductions in BRS noted in diabetic *vs* non-diabetic patients
Chan *et al*[32]	10 hypertensive ESRD patients receiving conventional hemodialysis were studied before and 2 mo after conversion to nocturnal hemodialysis Assessed BRS along with total arterial compliance	Spontaneous method	Improvement in BRS by nocturnal HD as compared to conventional HD Increases in BRS correlated with increases in total arterial compliance
Bavanandan *et al*[81]	105 non-dialysis CKD patients Baseline and follow-up of 42 mo Studied relationship with increasing degrees of uremia Recorded primary (death, dialysis, transplantation) and secondary (fatal and nonfatal cardiovascular events) outcome measures	Spontaneous method	Nondialysis dependent CKD patients have impaired BRS BRS is related to decreasing GFR A trend towards poorer prognosis in patients with impaired BRS
Studinger *et al*[33]	Juvenile study group with 14 HD patients, 14 RT and 14 controls BRS with HRV and carotid artery stiffness	Pharmacological and spontaneous method	BRS was markedly reduced in HD as compared to controls Carotid artery stiffness was higher in HD than controls and was inversely related to BRS HRV was also compromised in HD, and was directly related to BRS No significant differences in any of these variables between RT and controls Decreased baroreflex function in juvenile HD is partly due to loss of carotid artery elasticity and partly due to impaired heart rate variability. Renal transplantation may partly prevent impairment or improve compromised baroreflex function in young patients with ESRD
Chesterton *et al*[31]	40 HD patients Assessed BRS with arterial calcification and arterial stiffness indices	Spontaneous method	Reduced BRS in HD patients Reduced BRS is associated with increased vascular calcification and arterial stiffness
Lacy *et al*[82]	55 non-dialysis non-diabetic CKD patients BRS relationship with arterial stiffness and GFR	Spectral method	BRS reduced as renal disease severity increases Reduced GFR was correlated with increased PWV and decreased cardiac BRS Non-dialysis non-diabetic CKD patients with decreasing GFR have reduced cardiac BRS and increased large artery stiffness
Rubinger *et al*[35]	52 HD, 44 RT and 41 controls 16 patients before and after transplant BRS with HRV and BPV	Spontaneous method	In HD patients, BPV was increased, while HRV and BRS were markedly decreased as compared to controls RT was associated with normalization of BPV at short term (≤ 1 yr) and long term and with improvement of HRV at a long-term (> 1 yr) follow-up. After RT baroreceptor indices were significantly increased and returned to values similar to those of the control

Chesterton *et al*[77]	34 chronic HD Cross-sectional Relation with intra-dialytic hypotension	Spontaneous method	Impaired BRS predicts intra-dialytic hypotension
Kaur *et al*[34]	23 ESRD patients studied prospectively before and at 3 and 6 mo after RT BRS with central arterial stiffness and HRV and BPV	Spontaneous method	RT normalizes BRS in ESRD patients by 6 mo which follows the improvement in the central arterial stiffness

HD: Hemodialysis; RT: Renal transplantation; CKD: Chronic kidney disease; ESRD: End stage renal disease; GFR: Glomerular filtration rate; HRV: Heart rate variability; BPV: Blood pressure variability; BRS: Baroreflex sensitivity.

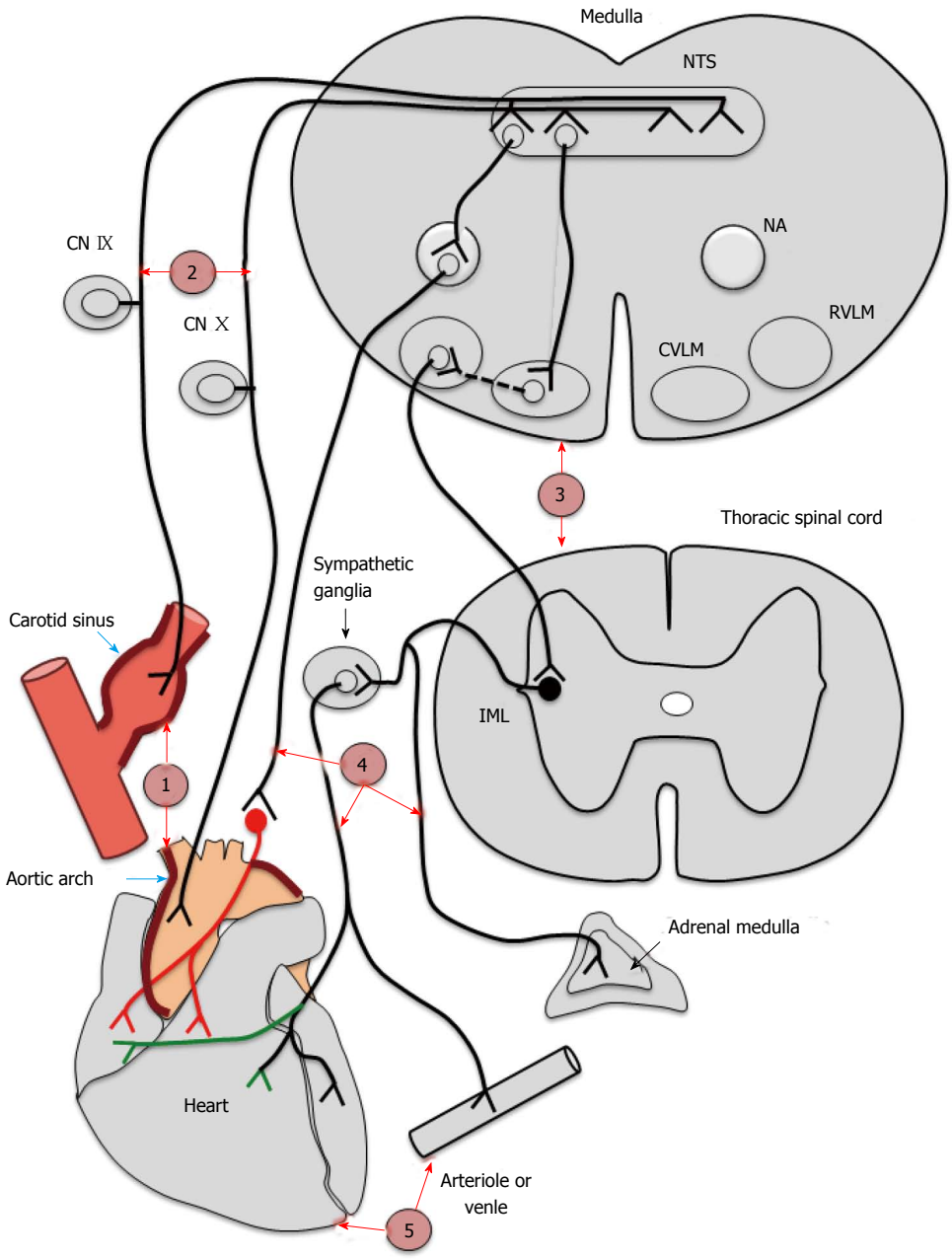

Figure 3 Probable levels of defect in baroreflex arc in chronic kidney disease. Depicts the different probable levels of defect in chronic kidney disease. Level 1 represents the baroreceptors affected by calcification of central arteries. Level 2, 3, 4 and 5 represents afferents (Ⅸ and Ⅹ nerves), centres, efferents and endorgans (heart and vessels) respectively. CN Ⅸ and CN Ⅹ: Cranial nerve Ⅸ and Ⅹ; NTS: Nucleus tractus solitarius; NA: Nucleus ambiguous; CVLM: Caudal ventrolateral medulla; RVLM: Rostral ventrolateral medulla; IML: Intermediolateral gray column.

Structure of the central arteries determines the deformation and thus the strain of baroreceptor endings with changes in blood pressure[93,94]. That is the reason for structural changes in the large arteries and increased arterial stiffness being considered the cardinal mechanism responsible for the reduced BRS and resetting of

baroreceptors in hypertension, atherosclerosis, and aging.

The current concept focuses on the functional mechanisms and thus the postulate that baroreceptor activity is not merely a manifestation of associated vascular strain.

Studies have identified various mechanisms involved in the modulation of the baroreflex arc. These are referred to as functional factors to differentiate them from structural changes. Based on their site of action, functional factors are categorized into two: (1) peripheral sensory mechanisms involving barorceptors and or sensory afferents; and (2) central mechanisms involving the neural areas coupling the afferent sensory stimuli to efferent autonomic responses[95].

Chapleau *et al*[96] studied the role of functional mechanism in baroreflex alteration in hypertensives and aged people. They examined on both cultured baroreceptor nodose neurons and isolated carotid sinus preparation of dogs and rabbits.

In their study[96], they found that peripheral sensory mechanisms include: (1) Lack of endogenous PGI2 and increase in free radicals and platelet aggregation which result in deranged baroreflex function in chronic hypertension and atherosclerosis; (2) Stretch activated channel and transient outward K current which are responsible for mechanoelectrical transduction and adaptation of baroreceptors respectively; and (3) Na-K pump inhibition, which occurs with fall in arterial pressure and leads to prompt (within minutes) reversal of chronic baroreceptor resetting in chronic hypertensive rabbits. The rapidity of response rules out structural change and could be due to functional change.

In their study[96], they have also commented on central mechanisms which include: (1) Loss of inhibition of sympathetic system and inefficient coupling of afferent stimuli to efferent response which could be attributed to reducedcentral arterial compliance and rapid frequency of baroreceptor discharge. It has been seen that 3 and a low frequency (< 3 Hz) of baroreceptor discharges sustain the reflex inhibition of sympathetic system; and (2) Defect in neural centres mediating the baroreflex arc. Authors have suggested that this may be the chief cause of the reduction in baroreflex functioning with aging.

Notionally, chronic kidney patients might have a similar structural and functional defect and functional changes may precede the structural changes unlike the present-day postulation but this concept has not been studied in CKD patients yet.

CONCLUSION

CKD patients have high cardiovascular mortality and morbidity. Baroreceptor function assessment is an independent predictor of cardiovascular risk. There are different methodological techniques and determinants of BRS. The underlying patho-physiology of baroreflex dysfunction is still unclear but probable defect seems to be central arterial stiffness in CKD patients resulting in dampened firing by baroreceptors (receptor level defect).

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MINIREVIEWS

Secondary amyloidosis in autoinflammatory diseases and the role of inflammation in renal damage

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Abstract

The release of proinflammatory cytokines during inflammation represents an attempt to respond to injury, but it may produce detrimental effects. The inflammasome is a large, multiprotein complex that drives proinflammatory cytokine production in response to infection and tissue injury; the best-characterized inflammasome is the nod-like receptor protein-3 (NLRP3). Once activated, inflammasome leads to the active form of caspase-1, the enzyme required for the maturation of interleukin-1beta. Additional mechanisms bringing to renal inflammatory, systemic diseases and fibrotic processes were recently reported, *via* the activation of the inflammasome that consists of NLRP3, apoptosis associated speck-like protein and caspase-1. Several manuscripts seem to identify NLRP3 inflammasome as a possible therapeutic target in the treatment of progressive chronic kidney disease. Serum amyloid A (SAA), as acute-phase protein with also proinflammatory properties, has been shown to induce the secretion of cathepsin B and inflammasome components from human macrophages. SAA is a well recognised potent activator of the NLRP3. Here we will address our description on the involvement of the kidney in autoinflammatory diseases driven mainly by secondary, or reactive, AA amyloidosis with a particular attention on novel therapeutic approach which has to be addressed in suppressing underlying inflammatory disease and reducing the SAA concentration.

Key words: Inflammation; Autoinflammatory disease; Chronic kidney disease; Interleukin-1; Dialysis; Caspase; Proteinuria; Amyloidosis; Nod-like receptor protein-3

Core tip: Inflammation may also negatively produce elevation of proinflammatory cytokines. Recently, attention was addressed to the formation of the intracellular inflammasome nod-like receptor protein-3 (NLRP-3) activating caspase-1, the enzyme required for the maturation of interleukin-1. IL1, in turn, regulate serum amyloid A, a major acute-phase with also proinflammatory properties. An interesting new scenario on the pathogenesis of renal diseases (namely ANCA-

associated glomerulonephritis vasculitis, urate-crystal nephropathy, contrast nephropathy, acute kidney injury, reactive systemic amyloidosis) and reactive systemic amyloidosis was opened, and NLRP3 inflammasome was recently identified as a possible therapeutic target in the treatment of chronic kidney disease.

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INTRODUCTION

Inflammation is a protective process, an attempt of the organism to respond to the harmful stimuli and at the same time to initiate the healing process for the tissue. Although the release of proinflammatory cytokines may have acute beneficial effects, chronic systemic elevation is likely to produce detrimental effects[1].

Inflammation is central to the pathogenesis of many renal diseases: The innate immune system, a first line defense against pathogens, is usually involved in the initiation and propagation of inflammation and moreover, chronic inflammation may contribute to progression of acute or chronic kidney disease (CKD).

NLRP3 mediated inflammation

Recently, several authors[2,3] seem to indicate additional mechanisms that may orchestrate renal inflammatory and fibrotic processes by the formation and activation of the intracellular inflammasome that consists of nod-like receptor protein-3 (NLRP-3), apoptosis associated speck-like protein (ASC) and caspase-1.

In the last few years several authors[2-9] underlined the importance of the NLRP3 inflammasome activation, the currently most fully characterized inflammasome, as an important player in renal injury. An interesting new scenario on the pathogenesis of renal diseases beyond the acquired knowledge in the rheumatologic field was opened, and NLRP3 inflammasome was recently identified as a possible therapeutic target in the treatment of progressive CKD.

Inflammasome: The inflammasome is a large, multi-protein complex that drives proinflammatory cytokine production in response to infection and tissue injury.

The best-characterized inflammasome is the NLRP3 inflammasome. On assembly of the NLRP3 inflammasome, post-translational processing and secretion of pro-inflammatory cytokines IL-1β and IL-18 occurs; in addition, cell death may be mediated *via* caspase-1[10].

Interleukin-1 (IL-1), previously known as endogenous pyrogen, osteoclast activating factor, catabolin, hemopoietin-1, lymphocyte activating factor, or epidermal-derived thymocyte activating factor, is produced as an inactive precursor form upon cell activation. Its release requires the activation of different molecules gathered under the name of "inflammasome".

The activation of inflammasome leads to the active form of caspase-1, the enzyme required for the maturation of IL-1. The release of IL-1 requires the activation of the cell by ATP through its P2X7 receptor that involvement of K^+ and Ca^+ channels and the action of a phosphatidylcholine-specific phospholipase. Necrotic cells produced by pressure disruption, but also hypoxic injury, uric acid crystals, bacterial toxins[11] or complement-mediated damage were capable of activating the NLRP3 inflammasome, triggered in part through ATP produced by mitochondria released by damaged cells (Table 1).

Some authors[12] indicate that the activation of the NLRP3 inflammasome requires two separate signals (Figure 1). The first signal, which can derive from Toll-like receptors, Tumor Necrosis Factor Receptors or IL-1R signaling, needs to activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) for the transcription and translation of the immature pro-forms of IL-1β and IL-18. As a second step, enzymatic cleavage is needed to secrete these pro-inflammatory ILs into the extracellular space.

The non-immune renal parenchymal cells do not seem to release IL-1β, as they do not express pro-IL-1β upon NF-κB activation[8], however, several reports document the expression and release of IL-18 from tubular epithelial cells (TECs)[13-15]. This would seem to indicate that the NLRP3 inflammasome and caspase-1 axis may also be in renal non-immune cells. Moreover, Zhang *et al*[16] using a confocal microscopy, documented NLRP3 and ASC to be expressed by glomerular podocytes.

Intrinsic renal cells express components of the inflammasome pathway: This is mostly prominent in TECs and, to a lower degree, in glomeruli. Several primary renal diseases and systemic diseases affecting the kidneys are associated with NLRP3 inflammasome/IL-1β /IL-18 axis activation. Most of the disorders studied have been acute inflammatory diseases: The disease spectrum includes ureteric obstruction, ischaemia reperfusion injury, glomerulonephritis, sepsis, hypoxia, glycerol-induced renal failure, and crystal nephropathy[17].

The German group from Munich recently described[7] the role of the NLRP3 inflammasome in oxalate nephropathy and found that calcium oxalate crystals kill TECs, which leads to the release of ATP and potentially other NLRP3-agonistic DAMPs that trigger IL-1β secretion by renal dendritic cells.

In addition, renal dendritic cells ingest oxalate crystals by phagocytosis and subsequent lysosomal leakage activates NLRP3. Acute oxalate nephropathy was significantly attenuated in NLRP3-, ASC- and caspase-1-deficient mice. Finally, acute oxalate nephropathy had been shown to be prevented by therapeutic IL-1 blockade with anakinra, a IL-1 receptor antagonist approved by the United States Food and Drug Administration for the treatment of rheumatoid arthritis. The results of this study suggest a potentially similar pathogenic role

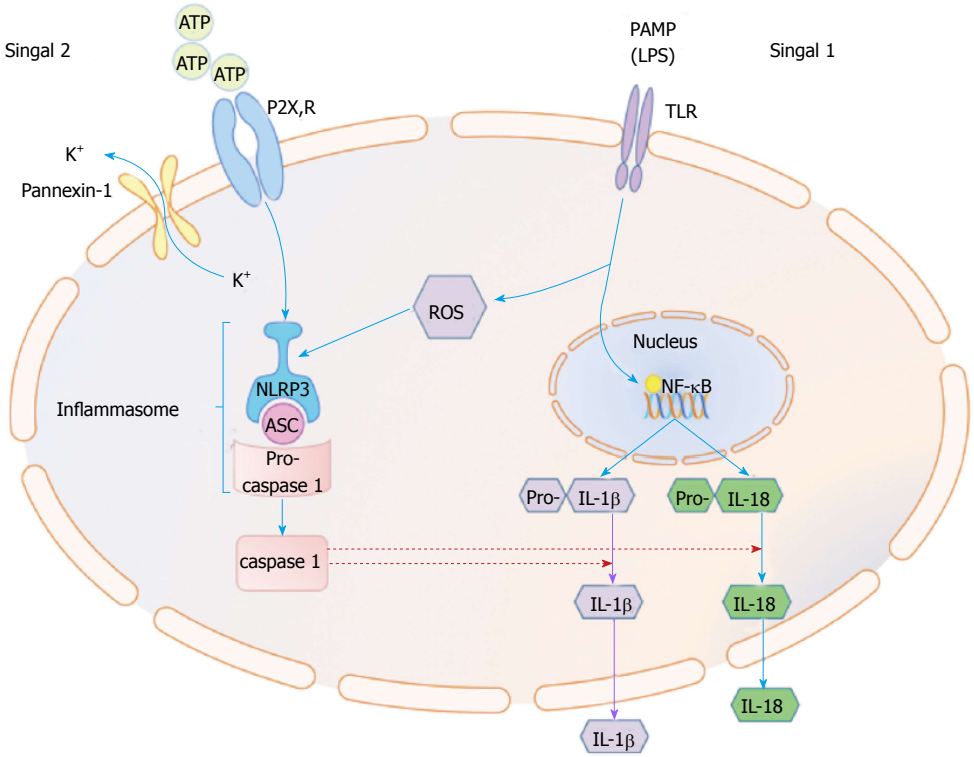

Figure 1 Model of nod-like receptor protein-3 inflammasome activation and the role of the nod-like receptor protein-3 inflammasome in the two-step activation of interleukin-1β and interleukin-18[10,12]. Activation of the NLPR3 inflammasome requires two signals. Signal 1: Activation of TLRs, IL-1Rs and TNFRs induces the transcription and translation of NF-κB to produce pro-forms of IL-1β and IL-18; Signal 2: Enzymatic cleavage by (caspase-11-driven) caspase-1 to secrete mature cytokines, IL-1β and IL-18. ROS: Reactive oxygen species; TLR: Toll-like receptor.

of the NLRP3 inflammasome in other crystal-related nephropathies such as cast nephropathy, contrast nephropathy, acute kidney injury (AKI) in rhabdomyolysis or urate nephropathy[17].

The role of the NLRP3 inflammasome arthritis in urate crystal-induced is well described[18] and the block of IL-1 may be considered a good therapeutic option in patients with gouty arthritis and renal failure[19].

Moreover, other authors reported that the upcoming data on the NLRP3 inflammasome support the evolving danger signaling concept of renal inflammation[20]. More recently Schreiber *et al*[21] described their experience in antineutrophil cytoplasmic antibodies (ANCA)-activated phagocytes that cause vasculitis and necrotizing crescentic glomerulonephritis (NCGN). The authors supposed that ANCA-induced phagocyte NADPH oxidase generated tissue-damaging reactive oxygen species that restrains inflammation, downregulated caspase-1, thereby keeping the inflammasome in check, reducing IL-1 generation and limiting ANCA-induced inflammation. The authors concluded that IL-1 receptor blockade by anakinra might provide a promising strategy in NCGN. More than 25 years ago, even in patients on hemodialysis, it was shown that the involvement of monocyte activation brings to the release of IL-1 and related cytokines, as already reported in 1988 by Dinarello[22].

Mulay *et al*[7] experimentally showed in mice that renal CaOx crystal deposition was associated with diffuse neutrophil infiltrates and tubular necrosis mainly at the inner stripe of the outer medulla, as demonstrated by the disintegration of TECs and granular casts in tubular lumen. The structural alterations of oxalate nephropathy were associated with renal failure[4]. Clodronate liposome was used in WT mice or diphtheria toxin in CD11c DTRg mice to demonstrate that CaOx-induced intrarenal IL-1 secretion originated from the intrarenal network of interstitial mononuclear phagocytes.

On hypothesizing that therapeutic blockade of IL-1 might be able to interfere with this pathomechanism and protect against renal failure, the authors[2] used anakinra: Intraperitoneal injection of anakinra dose-dependently reduced tubular injury and neutrophil recruitment and improved renal excretory function during oxalate nephropathy in mice. The authors concluded that IL-1 mediated inflammation and tissue damage in kidney injury induced by CaOx crystals and thus IL-1 blockade protected from renal failure in oxalate nephropathy in mice.

Both experimental and human studies show a detrimental role for NLRP3 in the development of acute and chronic tubule-interstitial disease[6]. To confirm this Duewell *et al*[23], using a novel microscopic technique, a combination of laser reflection and fluorescence confocal microscopy to identify in mice crystalline materials and immune cells, recently reported that minute cholesterol crystals were present in early diet-induced atherosclerotic lesions and that their appearance coincided with

Table 1 Pathogen associated molecular pattern and damage associated molecular pattern that trigger nod-like receptor protein-3 activation[12]

Type	Molecule/molecular pattern
PAMP	Leptospiral interrogans/glycolipoprotein
	Influenza
	Streptococcus pyogenes/streptolysin O
	Staphylococcus aureus/alpha hemolysin
DAMP	ATP
	Nigericin
	Histones
	U1snRNP ribonucleoprotein
	dsDNA/nucleosomes
	MSU crystals
	Uromodulin
	Biglycan
	Silica
	Alum
	Calcium oxalate
	Asbestos
	Amyloid-β
	Hemazoin
	Hyaluronan

PAMP: Pathogen associated molecular pattern; DAMP: Damage associated molecular pattern; ROS: Reactive oxygen species/oxidative stress; PAMP: Pathogen-associated molecular pattern; DAMP: Damage-associated molecular pattern; ATP: Adenosine tri phosphate; MSU: Mono sodium urate.

the first appearance of inflammatory cells.

To test whether cholesterol crystals could activate the release of IL-1β, the authors incubated lipopolysaccharides-primed human peripheral blood mononuclear cells with cholesterol crystals: Cholesterol crystals induced a robust, dose-responsive release of cleaved IL-1β in a caspase-1 dependent manner. The authors also demonstrated that cholesterol crystals also activated the NLRP3 inflammasome in phagocytes *in vitro* in a process that involved phago-lysosomal damage. Crystalline cholesterol acts as an endogenous danger signal and its deposition in arteries or elsewhere was an early cause rather than a late consequence of inflammation.

Most importantly, mice whose bone marrow-derived cells lacked NLRP3 inflammasome components, or IL-1 cytokines, were markedly resistant to developing atherosclerosis. The lesional area in the aorta of these mice was reduced on average by 69%, compared to chimeric LDLR-deficient mice that had wild-type bone marrow.

Amyloidosis: Amyloidosis is a disorder of protein folding in which normally whole or fragments of normally soluble proteins are deposited as abnormal, insoluble fibrils that disrupt tissue structure, so causing disease. In systemic amyloidosis the deposits may be present in the parenchyma of the viscera and tissues, causing progressive organ dysfunction leading patients to death. Systemic amyloidosis, fatal within 6 mo of diagnosis in up to 20% of patients, causes about one per thousand deaths in developed countries and remains an important unmet medical need. There are about 30 different types of amyloid in humans, characterized by the particular specific protein that forms the fibrils[24] (Figure 2).

Table 2 Over 30 proteins capable of amyloid formation have been identified

Immunoglobulin light chains in primary systemic amyloidosis
Ig heavy chain
Beta2-microglobulin in dialysis-associated arthropathy
Amyloid beta protein in alzheimer disease and down syndrome
Hereditary forms (including transthyretin, apolipoprotein A-Ⅰ and A-Ⅱ, gelsolin, lysozyme, fibrinogen a-alpha chain
Amyloid a in secondary amyloidosis

The core structure of all amyloid fibrils consists of antiparallel β-pleated sheets arranged with their long axes perpendicular to the long axis of the fibril. This structure specifically binds the histochemical dye, Congo-red, from alkaline alcoholic solutions, in an ordered molecular array which gives pathognomonic red-green birefringence when viewed in strong cross-polarized light. This is the gold standard for histological diagnosis of amyloid[24] (Table 2).

There are therefore both acquired and hereditary forms of amyloidosis. The most common form of systemic amyloidosis is the AL type. The international nomenclature comprises A for amyloidosis and the second and other letters identify the amyloid fibril protein, in this case L for monoclonal immunoglobulin light chains[24].

AL amyloidosis, formerly known as primary amyloidosis, is thus a complication of monoclonal gammopathy of any type ranging from myeloma through monoclonal gammopathy of uncertain significance, to the whole variety of B/plasma cell dyscrasias. It accounts for about 60% of all cases.

AA amyloidosis, formerly known as secondary or reactive systemic amyloidosis, is a complication of chronic inflammatory and infective diseases in which there is a sustained acute-phase response with overproduction of serum amyloid A (SAA) protein, a very sensitive and dynamic major acute-phase protein. Although becoming rare in the developed world due to greatly improved treatments for inflammatory arthritides, Crohn's disease, chronic infection, *etc.* AA amyloidosis is still a fairly common disease in medicine department and nephrologist's counseling may be required for the detection of proteinuria or renal failure[25].

In the past ten years, thanks to more aggressive treatment schedules and to the increasing availability of anti-TNF treatments, some authors[26,27] report that the incidence of AA amyloidosis in chronic arthritides has slowly decreased.

This has led to a relative increase in the rate of other conditions that are well-recognized to significantly associate with AA, such as Crohn's disease, hereditary periodic fevers, malignancies, systemic vasculitides and diseases predisposing to recurrent infections, including

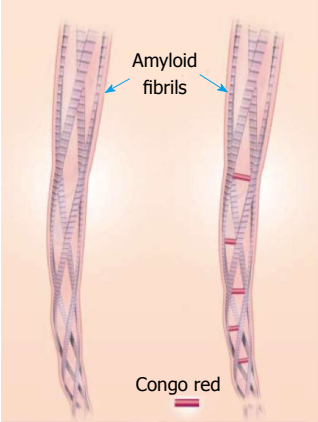

Figure 2 Structural features of amyloid[25].

cystic fibrosis, bronchiectasis, epidermolysis bullosa, cyclic neutropenia, acquired or inherited immunodeficiencies, injection-drug use and acne conglobata.

SAA is a major acute-phase protein present in serum but also shown to possess proinflammatory properties, as meaning that it can induce the release of cytokines from different cell types, including THP-1 monocytes, human neutrophils, and mast cells.

SAA is mainly produced in the liver under the regulation of IL-1, IL-6, and TNF-, but its expression has also been demonstrated in other cell types, including macrophages, endothelial cells, and smooth muscle cells.

Moreover, SAA has been shown to induce the secretion of cathepsin B and inflammasome components from human macrophages. As processing of SAA by cathepsin B may result in production of amyloidogenic SAA fragments: Experimentally, SAA has demonstrated to induce a strong expression of IL1β and TNFα in human macrophages[28].

SAA mediates its effect through activation of NLRP3 inflammasome: SAA is a potent activator of the NLRP3 inflammasome *via* a cathepsin B- and P2X7-dependent manner and is the first physiological proinflammatory mediator that can provide signals needed for expression of pro-IL-1 (as shown in Figure 1) and activation of the inflammasome cascade, resulting in activation of caspase-1 and secretion of mature IL-1β so resulting in formation of amyloidogenic fragments[28]. The conversion of the circulating soluble protein SAA into stable, highly ordered, amyloid fibrils that accumulate extracellularly causing organ damage is a multi-step process.

As an acute phase reactant secreted by the liver under the transcriptional control of IL-1 and IL-6, SAA increases up to 1000 fold following an inflammatory stimulation. If such stimuli persist, as occurs in several chronic diseases, SAA concentration may reach a critical threshold over which it becomes prone to aggregation. Moreover, the estimated ten years' survival was reported to be much higher in the patients with lower SAA levels, below 10 mg/dL[29].

A β-2m amyloidosis, so-called DIALYSIS-RELATED AMYLOIDOSIS, is a serious complication of long-term dialysis for end-stage renal failure in which β2-microglobulin, normally catabolized by the kidneys, is not adequately cleared and accumulates in the plasma, rising in concentration from its normal value of 1-2 mg/L to up to 70 mg/L[30].

All amyloid deposits have the feature to be largely ignored by the usually very efficient physiological mechanisms by which abnormal protein debris is cleared from the interstitial space in the tissues. Dead cells, effete matrix and structural proteins, blood cells and plasma proteins extravasated in injury, are normally rapidly cleared with no local or systemic clinical consequences. In contrast, although macrophages and giant cells are occasionally seen, especially around local rather than deposited as amyloid fibrils in and around bones and joints, causing pain, bone cysts and pathological fractures.

Here we will address our description on the involvement of the kidney in autoinflammatory disease driven mainly by secondary, or reactive, AA amyloidosis.

AA AMYLOIDOSIS

A clear example of renal involvement in autoinflammatory disease with amyloid A deposition may be considered the Muckle-Wells (MWS) disease associated with AA-amyloidosis. MWS is inherited as an autosomal dominant condition, meaning each child of a sufferer has a 50% chance of developing the syndrome.

MWS is a rare genetic autoinflammatory syndrome and the intermediate-severity form of cryopyrin-associated periodic syndrome (CAPS). As with other forms of this syndrome, it presents with recurrent episodes of fever, skin rash, joint pain, abdominal pain and conjunctivitis, but in addition sufferers typically develop a progressive sensorineural deafness and amyloidosis[9].

The protein affected in MWS is cryopyrin, produced by the NLRP3 gene located on chromosome 1. The gene is expressed in white blood cells (mainly neutrophils) and chondrocytes (cartilage cells). Cryopyrin is an essential component of the inflammasome, an intracellular protein complex involved in the innate immune system. The abnormal inflammasome in MWS allows unrestricted activation of the enzyme caspase-1, which in turn causes overproduction of active IL-1, switching on the inflammatory cascade in an uncontrolled manner[31].

MWS can have severe consequences due to chronic high levels of inflammation in the body. This can be life-threatening if generalized amyloidosis of the AA type develops, due to long-term buildup of amyloid protein products from the chronic inflammation in MWS. Organ damage results from the extracellular deposition of proteolytic fragments of the acute-phase reactant SAA as amyloid fibrils[9]. A sustained high concentration of SAA is the prerequisite for developing AA amyloidosis.

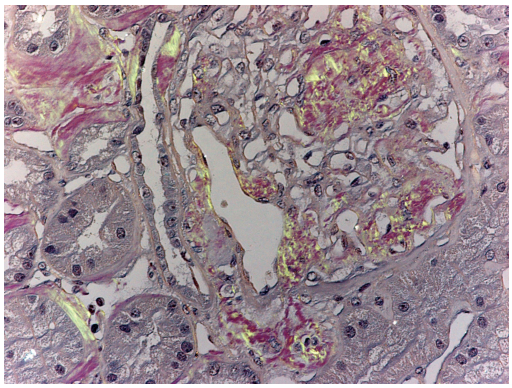

Figure 3 Renal biopsy: Amyloid fibrils bind congo red stain, yielding the pathognomonic apple-green birefringence under cross-polarized light microscopy[9].

The kidneys, liver and spleen are the main target organs of AA amyloid deposits (Figure 3).

In more than 90% of the patients proteinuria, nephrotic syndrome and/or renal dysfunction dominate the clinical picture at onset[9,24,26,27]. If not effectively treated, this disease invariably leads to end stage kidney disease[9] and renal replacement therapy, that are still associated with a poor outcome[27].

Over 25% of MWS patients have elevated serum amyloid, and at least 25% have amyloidosis. Serum AA testing is essential to follow, along with C-Reactive Protein (C-RP), Erythrocyte Sedimentation Rate (ESR) and other laboratory tests. Amyloidosis is also a risk to some patients affected by different types of CAPS, a group of autoinflammatory disorders characterized by recurrent episodes of systemic inflammation marked by fever, tissue inflammation, particularly of the joints and skin, and other constitutional symptoms, clinically defined by a spectrum of varying severity. Amyloidosis may be associated in familial cold autoinflammatory syndrome and in neonatal-onset multisystem inflammatory disease/chronic infantile neurological cutaneous and articular syndrome, but not so much as in MWS. Generalized amyloidosis is due to a permanent buildup of amyloid in the kidneys, liver and elsewhere, that can be fatal[32].

Clinical AA amyloidosis is typically preceded by many years of active inflammation before presenting, most commonly with renal involvement[33].

In AA amyloidosis renal dysfunction is reported to be the predominant disease manifestation. Mortality, amyloid burden, and renal prognosis all significantly correlated with the SAA concentration during follow-up. The risk of death was reported to be 17.7 times as high among patients with highest SAA concentrations. In the previously reported[25] largest study on AA amyloidosis involving 374 patients, the most frequent underlying disorder was inflammatory arthritis and only rare causes of AA amyloidosis included vasculitis, sickle cell anemia, malignant disease, epidermolysis bullosa, and cyclic neutropenia. Renal involvement was reported to be frequent: In 97% of patients, more than 500 mg of proteinuria per day were present or the serum creatinine concentration was more than 1.5 mg/dL. The relative risk of progression to end-stage renal failure was also increased among patients whose renal function was relatively worse at baseline, with an increase by a factor of 5 for each doubling of the baseline serum creatinine concentration ($P < 0.001$).

Fortunately, the cardiac involvement is not so frequent in AA amyloidosis and it is reported to be present in only 1 patient, and findings consistent with cardiac infiltration were present in only 2 among 224 patients who underwent echocardiography[34].

A worse renal outcome in patients with chronic sepsis or Crohn's disease was reported[28], possibly related to the high frequency of surgical intervention and administration of immunosuppressive drugs, probably due to greater severity of disease associated or not at increased risk of infection.

Therapy

Further studies need to elucidate whether persistent inflammation serves as a catalyst by sensing and converting the endothelium into a proinflammatory surface that makes the vasculature more vulnerable to the effects of other circulating risk factors. Such a scenario is supported by the strong documented association between inflammatory markers and endothelial dysfunction in patients with CKD.

Similarly, effective anti-inflammatory treatment, or whatever is needed to control the acute-phase response and maintain circulating SAA serum levels in the normal range, is life saving in AA amyloidosis[9,25,27]. Rigorous compliance with colchicine therapy for Familiar Mediterranean Fever (FMF) prevents and ameliorates AA amyloidosis even in patients who do not experience complete relief of symptoms. The key is to control SAA production, closely monitoring SAA serum levels in all patients with AA amyloidosis, and tailoring their treatment to keep these as low as possible. However, many patients are already in severe or end-stage organ failure when diagnosed with amyloidosis and new approaches are desperately needed to save them.

Treatment of AA amyloidosis has to be addressed in suppressing underlying inflammatory disease and reducing the SAA concentration as much as possible. If not effectively treated, this disease invariably leads to end stage kidney disease and renal replacement therapy, that are still associated with high mortality rate[25].

In a unpublished experience we observed in a female patient aged 40, affected by Chron disease with nephrotic proteinuria of 14 g/daily and CKD stage 3 secondary to renal AA amyloidosis histologically proven, the control of the baseline chronic bowel inflammatory disease with the monoclonal antibody adalimumab, a TNF-alfa inhibitor, significantly reduced the proteinuria levels up to 6 g/daily, while still remaining in the nephrotic range. The TNF-inhibitor therapy also reduced SAA levels from more than 4 mg/dL (normal values <

0.5) up to quite normal values (0.61 mg/dL).

Among AA amyloidosis therapy, some years ago interest was pointed on eprodisate, structurally similar to heparin sulfate, a glycosaminoglycan that is known to promote fibril assembly, inducing amyloid formation. Eprodisate, negatively charged, sulfonated molecule that is structurally similar to heparin sulfate and works by competitively inhibiting the interaction between SAA and glycosaminoglycans.

A RCT was conducted[35] enrolling 180 patients with AA amyloidosis-associated nephropathy; patients were treated with eprodisate or placebo for 24 mo: The authors reported that the treatment was associated with a 42% reduction in the risk of worsening renal disease (as measured by creatinine clearance) or death (CI: 0.37-0.93; P = 0.02), compared with placebo. Surprisingly, there was no significant difference in terms of the overall changes in proteinuria: A second phase Ⅲ trial is now ongoing.

Higher levels of aspecific laboratory inflammatory markers such as C-RP and sTNF are independently associated with faster rates of kidney function loss in CKD[36]. Pravastatin, a HMG-CoA reductase inhibitor, was reported to prevent loss of kidney function to a greater extent in CKD individuals with coronary artery disease (CAD) with greater evidence of inflammation, although this was of borderline significance. These data suggest that inflammation may mediate the loss of kidney function among subjects with CKD and concomitant CAD[37].

Some years ago, other authors experimentally found that inhibition of the isoprenoid pathway by another statin, lovastatin, resulted in a dose-dependent reduction of amyloid formed in mouse recombinant SAA produced in Escherichia coli, hypothesizing the isoprenoid metabolism as a potential target for prevention and treatment of AA amyloidosis[38].

More recently, Luo *et al*[39] studied the effects of another statin, rosuvastatin (RSV), and observed that, compared with controls, diabetic Sprague-Dawley rats showed severe metabolic disorder, cardiac dysfunction, fibrosis, disorganized ultrastructure, and excessive activation NLRP3 inflammasome, ASC, IL-1β and mitogen-activated protein kinases. The NLRP3 inflammasome was found activated in response to high levels of glucose. RSV was added and continued for 8 wk. The effect and underlying mechanisms of action of RSV in diabetic cardiomyopathy (DCM) and whether NLRP3 was a target for RSV in DCM, was studied. The authors concluded that, compared with diabetics rats alone, RSV experimentally ameliorated the overexpression of NLRP3 inflammasome and silencing NLRP3, ameliorated cardiac remodeling and dysfunction, so identifying RSV as a significant potential therapy *via* inhibition of NLRP3 inflammasome.

Due to the strong association between proinflammatory cytokines and complications common in ESRD, such as vascular calcification and wasting, the potential role of both general and targeted anticytokine treatment strategies in ESRD patients needs further evaluation[40]. Inflammation has to be considered an important target for pathogenetic interventions both in AKI and in progression of CKD, as recently suggested[41].

Therapeutic interventions that suppress inflammation and oxidative stress may address both short-term (dynamic) and long-term (structural) contributors to a decline in the GFR in patients with CKD and could possibly stabilize or even improve kidney function[9,42].

However, despite major technologic improvements in dialysis techniques, a lot of haemodialysis and peritoneal dialysis patients show serological evidence of an activated inflammatory response, as clearly indicated by increased circulating levels of non-specific markers of inflammation and proinflammatory cytokines such as IL-6.

Dialysis treatment save the lives of patients with ESRD but it does not cure the burden of clinical consequences related to uremic state, *i.e.,* the marked risk for atherosclerotic cardiovascular disease and inflammation. Renal transplantation (TPX) can be considered in selected patients progressing to ESRD, but unfortunately, it is a choice not offered to all ESRD patients due to the low number of transplants performed in some countries.

Novel treatments to control inflammation processes, and also to prevent progression of renal damage, are under development and anti-cytokine agents are becoming the mainstay of therapy to prevent and treat AA, including patients with FMF that do not respond or do not tolerate adequate colchicine dosages and targeting key molecular events in the fibrillogenesis process[43]; also the role of other drugs are in progress[44].

Unfortunately, control of fibril-protein production is not possible in some forms of amyloidosis and in others it is often slow. There is no therapy that directly targets amyloid deposits for enhanced clearance. However, all amyloid deposits contain the normal, non-fibrillar plasma glycoprotein, serum amyloid P component (SAP).

Other authors[45] showed that administration of anti-human-SAP antibodies to mice with amyloid deposits containing human SAP triggers a potent, complement-dependent, macrophage-derived giant cell reaction that swiftly removes massive visceral amyloid deposits without adverse effects. Interestingly, the authors found that a combination of a drug that depletes circulating SAP and an antibody that targets residual SAP within the deposits results in clearance of amyloid deposits. A humanized version of the anti-SAP antibody has been developed with a view to clinical evaluation of this dual approach, hypothesizing this combined therapy to eliminate amyloid deposits.

IL-1 blockade

Clinical observations to date suggest that although IL-1 plays a key role in activation of the innate immune system, blockade of this cytokine appears to have few adverse effects. Anti-IL-1 therapy appears to

increase the risk of infection only marginally, and there is no clear evidence for increased risk of malignancy, despite lymphoma and other types of cancer have been reported in children treated with TNF blockers, often when along with certain other drugs (such as azathioprine or 6-mercaptopurine). Safety block of IL-1 after 12 mo after renal TPX was reported[46] also in a renal transplanted patients affected by MWS with systemic amyloidosis treated with triple immunosuppressive drug regimen and at the same time canakinumab: No flares of MWS was observed during this period.

In our experience we did not observe increased hospitalization rate due to infections or malignancy in two patients affected by Muckle Wells syndrome treated with IL-1 blockers who had been followed for over three years[9].

CONCLUSION

The release of proinflammatory cytokines during inflammation represents an attempt to respond to injury, but it may produce detrimental effects. The best-characterized inflammasome is the NLRP3 that, once activated, leads to the active form of caspase-1, the enzyme required for the maturation of IL-1β. SAA, as acute-phase protein with also proinflammatory properties, is a well recognized potent activator of the NLRP3. Additional mechanisms bringing to renal inflammatory, systemic diseases and fibrotic processes, resulting in kidney insufficiency were recently reported, *via* the activation of the inflammasome.

Currently, treatment options in amyloidosis rely on reducing the supply of the precursor protein and thus depend absolutely upon accurate typing of the amyloid. Intercalating agents able to induce physical disruption of the fibrillar structure of the native fibrils, once mature fibrils have been deposited, are under study in some types of non-AA amyloidosis, hence producing an intermediate: It so resulting to be more readily available for enzymatic degradation.

The administration of anti-human SAP antibodies[47] to mice with amyloid deposits containing human SAP triggers a potent, complement-dependent, reaction that swiftly removes massive visceral amyloid deposits without adverse effects. These promising results achieved in mouse models based on intermediary metabolism may not be extended to humans, so specifical trials are needed to test this hypothesis also in humans.

The role of statins is a new aspect targeted towards NLRP3 and not only in ameliorating dyslipidemic profile: Treatment with statins may represent a promising further test for this well-known class of drugs beyond the CV risk reduction, mediated by the reduction of lipidic profile[39].

Recently, great interest is growing on the role of NLRP3 inflammasome that incorporates several signals of tissue injury, infectious or non-infectious, and consequently brings, *via* the activation of caspase-1, to the secretion of the pro-inflammatory cytokines IL-1β and IL-18. Block of the IL-1 system seems to be a fascinating option to counteract caspase activation and reducing IL-1 levels and consequently also SAA levels, that appear to be dramatically reduced within normal values even in patients with border line levels up to thousands of times. The mainway is to control the primary cause of inflammation and IL-1 blockers have demonstrated in rheumatologic field to be really effective and safe, even when associated to important immunosuppressant therapy, such as in kidney transplant patients. Moreover, a possible targeted intervention of IL-1 receptor blockade, even in active vasculitis, was recently suggested[22].

Several question points remain open, such as whether it is right to consider IL-1 block as target for treating CKD. And also if it is really the NLRP3 inflammasome a gauge of kidney injury damage or if we can specifically target the NLRP3 inflammasome for therapeutic intervention, as recently postulated by other authors[12].

The direct involvement of NLRP3 in kidney disease has not been demonstrated yet, despite recently several manuscripts address a reasonable suspicion about it. Moreover, a deeper knowledge on the role of NLRP3 inflammasome and of reactive AA amyloidosis in renal diseases is requested. Whether blocking IL-1 is really effective in delaying the progression of renal damage has yet to be demonstrated by large trials, despite, at the moment, the high costs severely limit the use of such drugs.

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Role of calcium in polycystic kidney disease: From signaling to pathology

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited monogenic kidney disease. Characterized by the development and growth of cysts that cause progressive kidney enlargement, it ultimately leads to end-stage renal disease. Approximately 85% of ADPKD cases are caused by mutations in the *PKD1* gene, while mutations in the *PKD2* gene account for the remaining 15% of cases. The *PKD1* gene encodes for polycystin-1 (PC1), a large multi-functional membrane receptor protein able to regulate ion channel complexes, whereas polycystin-2 (PC2), encoded by the *PKD2* gene, is an integral membrane protein that functions as a calcium-permeable cation channel, located mainly in the endoplasmic reticulum (ER). In the primary cilia of the epithelial cells, PC1 interacts with PC2 to form a polycystin complex that acts as a mechanosensor, regulating signaling pathways involved in the differentiation of kidney tubular epithelial cells. Despite progress in understanding the function of these proteins, the molecular mechanisms associated with the pathogenesis of ADPKD remain unclear. In this review we discuss how an imbalance between functional PC1 and PC2 proteins may disrupt calcium channel activities in the cilium, plasma membrane and ER, thereby altering intracellular calcium signaling and leading to the aberrant cell proliferation and apoptosis associated with the development and growth of renal cysts. Research in this field could lead to the discovery of new molecules able to rebalance intracellular calcium, thereby normalizing cell proliferation and reducing kidney cyst progression.

Key words: Autosomal dominant polycystic kidney disease; Calcium signaling; cAMP; Cell growth; Non-capacitative calcium entry

Core tip: In the present article, we discuss: (1) the regulation of calcium signaling in the primary cilia of autosomal dominant polycystic kidney disease (ADPKD) cells and the downstream processes that lead to cystogenesis; (2) how calcium impairment promotes cell proliferation by activating different signaling pathways; (3) the activity of non-capacitative calcium entry channels, which in PKD1-silenced cells stimulates cell growth by Ca^{2+} oscillations and nuclear factor of activated T-cells activation, highlighting new findings showing the role of polycystin-2 in calcium oscillations; (4) the impairment of intracellular calcium signaling associated with apoptosis; and (5) the use of calcium channel blockers and calcium modulators in the treatment of ADPKD.

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INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited pathology of the kidneys, having an incidence of 1:500-1:1000 individuals. It accounts for roughly 10% of cases of end-stage renal disease[1,2], which results from the progressive bilateral development and expansion of fluid-filled cysts arising from the de-differentiation of renal tubule epithelial cells[1]. ADPKD is caused by the mutation of two genes: *PKD1*, which accounts for 85% of cases, and *PKD2*, associated with the remaining 15% of cases[1]. In ADPKD, the focal cyst development in the kidneys seems to be associated with a somatic second hit brought on by either loss of heterozygosity or other mutations in renal cyst lining epithelial cells[3,4]. *PKD1* and *PKD2* genes encode for polycystin-1 (PC1) and polycystin-2 (PC2) proteins, respectively[1,5]. PC1 is an integral membrane receptor with a large extracellular region consisting of a variety of domains involved in cell-cell and cell-matrix interactions. It also bears 11 transmembrane domains, and a short cytoplasmic segment containing motifs involved in signal transduction[1]. PC2, on the other hand, is an integral transmembrane protein mainly localized to the endoplasmic reticulum (ER); it is anchored to cell membranes by six transmembrane regions, and has two cytoplasmic N- and C-terminal tails. PC2 functions as a nonselective cation channel that transports calcium, and shows significant homology with transient receptor potential (TRP) channels[5-8]. Indeed, the polycystins and their homologous proteins are considered a new subfamily of TRP channels, and accordingly known as TRP polycystic proteins[1].

An interaction between PC1 and PC2 forms the so-called polycystin complex. This is mainly confined to the primary cilia of kidney epithelial cells, where it acts as a flow sensor, triggering intracellular calcium release *via* the activation of the PC2 channel in response to fluid-flow changes. Disruption of this complex impairs intracellular calcium influx, and leads to the development and expansion of kidney cysts[1,9,10].

Polycystins are able to regulate calcium channel activity not only in the cilia, but also in other cellular compartments, including the plasma membrane and ER. Indeed, PC1 and PC2 co-assembly has been seen to generate a cation-permeable current through the plasma membrane[11], and PC1 and PC2 are known to regulate intracellular calcium release in the ER through their interaction with the inositol 1,4,5-trisphosphate receptor (IP_3R)[12-14]. In this context, PC2 enhances calcium release from the ER by stimulating the activity of the IP_3 receptor, while PC1 inhibits this process by reducing PC2-IP_3R interaction *via* a mechanism involving the stromal interaction molecule-1 (STIM1) and the PI3K/Akt pathway[12,15]. PC1 can also regulate other types of calcium channels, including non-capacitative calcium entry (NCCE) channels, which are able to generate intracellular calcium oscillations[16]. PC2, on the other hand, regulates intracellular calcium release by either interacting with the calcium channels TRPC1 and TRPV4 on the plasma membrane and in primary cilia, and/or through an association with ryanodine and IP3 receptors in the ER[8,17-19]. Moreover, PC2 appears to be able to generate a non-specific voltage-dependent cation current in native HEK293 kidney cells. This current is strongly associated with PC2 activity, and is completely abolished by the depletion of PC2 protein[20].

Taken together these findings suggest that PC1 and PC2 may affect calcium influx from different cellular compartments, including cilium boundaries (cilioplasm), plasma membrane and ER. Dysregulation of calcium signaling due to loss of polycystin function causes the aberrant activation of different pathways associated with abnormal cell proliferation and fluid secretion, thereby leading to the development and expansion of kidney cysts. However, the cascade of events that occur between polycystin dysfunction and kidney cyst formation in ADPKD is not yet fully understood.

In this review, we discuss the impact of polycystin loss of function on calcium signaling, which may alter different pathways associated with the cell growth and apoptosis that are a typical hallmark of ADPKD. Moreover, we report the potential effects of calcium dysregulation on kidney cyst formation and progression. Finally, we also discuss the state of the art in calcium channel modulators, able to restore normal calcium release and therefore appealing targets for ADPKD treatment.

ROLE OF THE POLYCYSTIN COMPLEX IN PRIMARY RENAL CILIA

It is well known that PC1 and PC2 co-localize in the

primary cilia of kidney epithelial cells, performing a mechano-sensor function by transducing calcium signals in response to changes in tubular fluid flow. Loss or dysfunction of either PC1 or PC2 causes the inability of cells to sense mechanical stimuli due to bending of the cilia, which leads to abnormal cell morphology and polarity, and thereby contributes to renal cyst formation[10,21,22]. The calcium signaling triggered by fluid shear stress initiates in the primary cilia through PC2-dependent calcium release. This is initially confined to the cilioplasm, but, through the ryanodine receptor, the same calcium signal subsequently activates a cytosolic calcium response that induces calcium influx from intracellular stores[23].

PC2, as mentioned above, is also able to interact with the transient potential receptor channels TRPC1 and TRPV4. PC2 and TRPC1 assemble to form a hetero-multimeric channel, not associated with PC1, which is activated in response to G-protein-coupled receptor stimulation, and shows a pattern of single-channel conductance distinct from that of the individual PC2 and TRPC1 channels[24]. Direct or indirect activation of the PC2/TRPC1 complex, either by cilium bending or through the activation of plasma membrane GPCRs, may affect the mechano-transduction of cilium-associated calcium signals[24]. PC2 can also form a heteromeric channel complex with TRPV4. This complex displays molecular mechano-sensor properties, being able to generate flow-induced calcium influx, which seems to be abolished by the depletion of TRPV4 channel in renal epithelial cells[18]. It is also plausible that polycystins cooperate with other proteins located in the cilium, such as cystin, polaris, inversin, and kinesin-Ⅱ, as defects in these proteins may lead to the formation of kidney cysts[21,25].

In the primary cilia, PC1/PC2, PC2/TRPC1 and PC2/TRPV4 complexes regulate the calcium signaling activated by cilium deflection due to changes in fluid flow. Surprisingly, however, depletion of TRPC1 and TRPV4 is not associated with cyst formation, despite it altering ciliary calcium signaling. Hence, the impairment of ciliary calcium signaling alone is not sufficient to trigger kidney cyst development, a process which, instead, seems to be closely linked to the activity/function of PC1 and PC2 proteins. In fact, a recent study has shown that ablation of the cilia in both PC1- and PC2-deficient cells reduces cyst growth, suggesting that the loss of cilia may cause milder cyst progression than in the cilia-equipped ADPKD cells[26]. Moreover, cilia-dependent cyst growth is not associated with the activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase, mammalian target of rapamycin (mTOR) or cyclic adenosinemonophosphate (cAMP) pathways[26]. As a whole, these findings suggest that the polycystins govern ciliary signaling by an unknown mechanism when the normal kidney epithelial cell phenotype is maintained. In ADPKD, the inactivation of polycystins alters cilia-dependent signaling, thereby promoting the formation of the characteristic kidney cysts.

CALCIUM SIGNALING AND CELL PROLIFERATION IN ADPKD CELLS

ADPKD is strongly associated with the altered cell proliferation of cystic kidney epithelial cells that represents a typical hallmark of the disease. The disruption of calcium signaling associated with PC1 and PC2 deficiency could be the primary event behind the increased cell growth seen in ADPKD. Indeed, we do know that calcium restriction in ADPKD cells causes cAMP-dependent activation of the B-Raf/mitogen-activated protein kinase kinase (MEK)/extracellular-signal-regulated kinases (ERK) pathway, which results in increased cell growth[27]. Moreover, this reduction in intracellular calcium levels also inhibits the activity of AKT kinase, a negative regulator of B-Raf[27]. In cystic cells, normal growth can be restored by increasing their cytosolic calcium concentration, which increases AKT activity and inhibits cAMP-dependent B-Raf/ERK activation[28].

Low intracellular calcium levels may also stimulate the activity of the ciliary calcium-sensitive adenylyl cyclases AC5 and AC6, as well as the plasma-membrane-anchored AC6, leading to the elevation of cAMP[29,30]. Therefore, loss of polycystin function may promote the activity of AC5/6 by reducing intracellular Ca^{2+} release from the cilia, ER and plasma membrane[29,30] (Figure 1). Indeed, it has recently been reported that the double knockout of *PKD1* and *AC6* genes decreases cystogenesis, improves renal function and increases survival in a mouse model of ADPKD. These improvements in renal function occur through a reduction in cAMP levels and inhibition of the B-Raf/MEK/ERK pathway, suggesting that AC6 could be a key mediator of cyst formation in ADPKD[31]. In addition, cAMP elevation may activate the cAMP-response element-binding protein, which promotes cell proliferation in an epidermal growth factor receptor (EGFR)-activation-dependent manner by stimulating expression of the EGF-like peptide amphiregulin[32]. EGFR signaling is dependent upon a mechanism involving the sequential activation of Ras, Raf-1, MEK and ERK. It can converge on the same pathway activated by cAMP, thereby leading to activation of the ERK kinases that promote cell proliferation in ADPKD cells[33] (Figure 1). Furthermore, the abnormal activity of mTOR kinase has been observed to contribute to increased cell proliferation and cyst formation in ADPKD cyst-lining epithelial cells. In normal kidney epithelial cells mTOR activity is inhibited by PC1, which interacts with TSC1/TSC2, an inhibitory complex of mTOR, preventing its inactivation. Conversely, in ADPKD cells polycystin dysfunction promotes mTOR activation by inhibition of the TSC1/TSC2 complex, through a mechanism involving the cAMP-dependent B-Raf/ERK pathway[34,35] (Figure 1).

The expression of full-length PC1 has been shown to inhibit intracellular calcium release in response to ATP in Madin-Darby canine kidney (MDCK) cells, in a mechanism that involves the interaction of STIM1 with

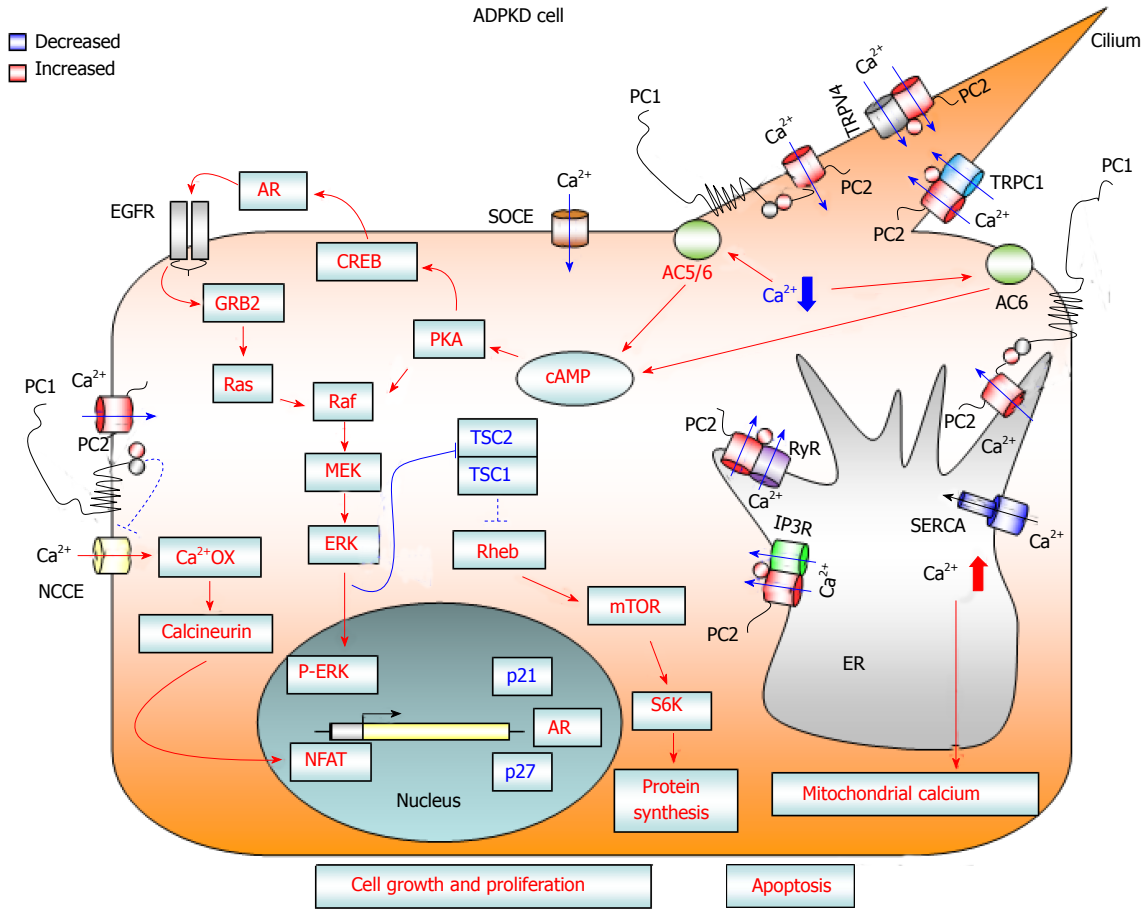

Figure 1 Diagram showing calcium-dependent dysregulated signaling pathways that promote cell proliferation and apoptosis in autosomal dominant polycystic kidney disease cells. Loss of PC1 and/or PC2 function causes a reduction in cytosolic calcium influx from three different cellular compartments: (1) the primary cilium after mechanical stimuli; (2) the endoplasmic reticulum, in an IP3R- and RyR-dependent manner; and (3) the plasma membrane, through a reduction in SOCE channel activity. The reduced concentration of cytosolic calcium may activate Ca^{2+} sensitive adenylyl cyclases 5 and 6, leading to a rise in cAMP. Increased levels of cAMP cause the activation of B-Raf/MEK/ERK and CREB/AREG/EGFR pathways, as well as stimulating mTOR signaling, through the active form of ERK kinases that inactivate the TSC1/TSC2 complex. Moreover, deficiency of PC1 and/or PC2 enhances the activity of NCCE channels, which, by increasing calcium oscillation frequency, results in the activation of the transcription factor NFAT. The abnormal activation of these signaling pathways promotes cell proliferation and kidney cyst formation. In addition, the reduction in Ca^{2+} influx from the ER to the cytosol caused by a deficiency in PC2 channel activity brings about an imbalance in ER calcium concentration, resulting in ER Ca^{2+} overload. The increased ER calcium concentration sensitizes kidney cystic cells to apoptotic stimuli by abnormal ER calcium release, which may induce mitochondrial damage and thereby lead to cytochrome C release and activation of apoptosis. AC 5/6: Adenylyl cyclase 5/6; AR: Amphiregulin; Ca^{2+} OX: Calcium oscillations; cAMP: Cyclic adenosine monophosphate; CREB: cAMP response element binding transcription factor; EGFR: Epidermal growth factor receptor; ER: Endoplasmic reticulum; ERK: Extracellular-signal-regulated kinases; GRB2: Growth factor receptor-bound protein 2; IP3R: Inositol 1,4,5-trisphosphate receptor; MEK: Mitogen-activated protein kinase kinase; mTOR: Mammalian target of rapamycin; NCCE: Non-capacitative calcium channel entry; NFAT: Nuclear factor of activated T-cells; PKA: Protein kinase A; PC1: Polycystin-1; PC2: Polycystin-2; S6K: Ribosomal S6 kinase; Raf: Rapidly accelerated fibrosarcoma kinase; Ras: Rat sarcoma viral oncogene homolog family; Rheb: Ras homolog enriched in brain; RyR: Ryanodine receptor; SERCA: Sarcoplasmic endoplasmic reticulum calcium ATPase; SOCE: Store-operated calcium channel entry; TRPC1: Transient receptor potential channel 1; TRPV4: Transient receptor potential cation channel subfamily V member 4; TSC: Tuberous sclerosis complex.

IP_3R, and reduces the association between PC2 and the IP_3 receptor[15]. Moreover, PC1 seems able to regulate intracellular calcium release and PC2-IP_3R-STIM1 interaction through the PI3K/Akt signaling pathway[15]. The exogenous expression of the C-terminal fragment of PC1 (PC1-Cter) could function as a dominant negative effector, causing an increased intracellular calcium release in response to ATP treatment, as seen in HEK-293 cells[36]. Furthermore, PC1-Cter-expressing cells not only exhibit increased levels of basal calcium, but also show enhanced cell proliferation, which is associated with the activation of ERK kinases[37]. Consistently, the transfection of HEK-293 cells with the C-terminal tail of PC1 has been observed to cause an increase in both basal and intracellular calcium release, leading to the activation of the nuclear factor of activated T-cells (NFAT)[38]. Moreover, NFAT activation, associated with increased cell proliferation in HEK-293 cells, is also observed after the downregulation of PC1 by RNA interference[16]. NFAT activation occurs through a rise in the frequency of intracellular calcium oscillations, caused by the increased activity of NCCE channels[16] (Figure 1). In HEK293 cells, these calcium oscillations can be increased by either reduced or undetectable levels of PC1, but are only induced by the absence of PC2 (Figure 2A). Normal calcium oscillations can be restored in *PKD1*-deficient cells *via* the reintroduction of mouse wild-type PC1, and in PKD2 knockout cells *via* the

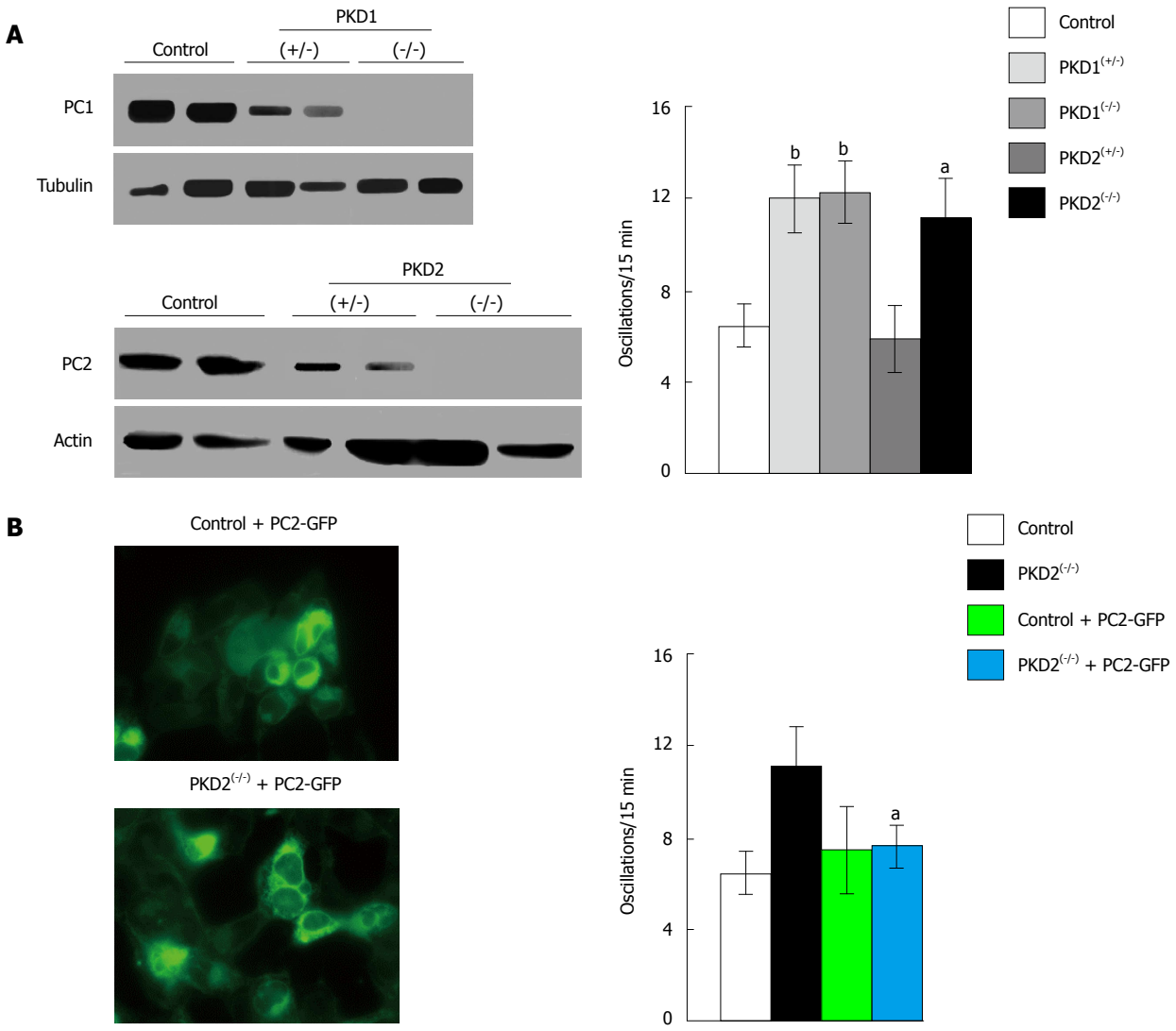

Figure 2 Downregulation of *PKD1* and *PKD2* genes increases fetal bovine serum-induced calcium oscillations in HEK293 cells. A: The stable transfection of HEK293 cells with plasmids containing specific anti-PKD1 and anti-PKD2 sequences causes a partial (+/-) or complete (-/-) downregulation of PC1 and PC2 expression compared with HEK293 cells stably transfected with scramble sequences (control). *PKD1* and *PKD2* gene silencing was evaluated by Western blotting using anti-PC1 and anti-PC2 antibodies. Calcium oscillations were increased in both partially (+/-) and fully (-/-) cells silenced for the *PKD1* gene, as well as in fully (-/-) PKD2-silenced cells, as compared with scramble-treated cells (control). The number of oscillations/15 min were: 12 ± 1.5 in PKD1$^{(+/-)}$ cells, 12.2 ± 1.42 in PKD1$^{(-/-)}$ cells and 11.13 ± 1.79 in PKD2$^{(-/-)}$ cells, *vs* 6.39 ± 1.09 in control cells ($^{b}P < 0.01$; $^{a}P < 0.05$); B: The expression of full-length exogenous PC2 fused with GFP in PKD2$^{(-/-)}$ cells restores normal calcium oscillations (11.13 ± 1.79 oscillations/15 min in PKD2$^{(-/-)}$ cells *vs* 7.72 ± 1.07 in PKD2$^{(-/-)}$ cells transiently transfected with PKD2-GFP cDNA; $^{a}P < 0.05$). Western blotting, oscillation recording and cell imaging were performed as previously reported[16]. Data, obtained from three different experiments analyzing at least 45 cells for every HEK293 clone, are represented as mean ± standard deviation. Analysis of data was performed using Student's t test, and differences were considered significant at a value of $P < 0.05$. PKD: Polycystic kidney disease; HEK293: Human embryonic kidney cells; GFP: Green fluorescent protein; PC: Polycystin.

transfection of full-length PC2 (reference[16] and Figure 2B). These findings suggest that PC1 could negatively regulate NCCE channels in a mechanism involving PC2 expression.

Taken as a whole, the evidence above suggests that the increased cell proliferation, fluid secretion and kidney cyst development seen in ADPKD may arise due to either the loss of polycystin complex function or an imbalance in the PC1/PC2 ratio causing intracellular calcium changes that trigger the B-Raf/MEK/ERK signaling cascade, as well as mTOR and NFAT pathways (Figure 1). However, the different effects on intracellular calcium concentration and downstream events observed with PC1 fragments and full-length PC1 expression suggest that further investigations are needed to clarify the function of polycystins in the regulation of calcium signaling.

CALCIUM SIGNALING AND APOPTOSIS IN ADPKD CELLS

In ADPKD, cyst formation and expansion rely on multiple mechanisms, including apoptosis, whose levels are higher in kidney cells from patients with ADPKD with respect to healthy individuals[39]. As apoptosis is one of the multiple cellular processes regulated by calcium

signaling, this increase in apoptosis may be associated with abnormal intracellular calcium influx. Indeed, it has been demonstrated that cell sensitivity to apoptotic stimuli can be enhanced by calcium accumulation in the ER of renal epithelial cells deprived of functional PC2[40]. Conversely, expression of the PC2 protein, which functions as a calcium channel, inhibits apoptosis by lowering ER calcium levels[40]. Therefore, polycystin dysfunction appears to bring about an imbalance in ER calcium concentration through a reduction in the activity of the PC2 channel, causing calcium overload in the ER. This increase in ER calcium concentration, and its subsequent release, sensitizes cystic kidney cells to apoptotic stimuli. The excess calcium released from the ER is absorbed by the mitochondria, potentially causing damage that may lead to the release of cytochrome C, which activates the programmed cell death (Figure 1). In light of these findings, it seems that PC2 may function as an anti-apoptotic calcium channel in kidney epithelial cells[40]. Likewise, programmed cell death in kidney cells may be also regulated by PC1. In fact, apoptosis is prevented in MDCK cells, through the activation of the phosphatidylinositol 3-kinase/Akt signaling pathway, by the expression of full-length PC1[41,42].

CALCIUM CHANNELS AS A TARGET FOR ADPKD THERAPY

Drugs able to inhibit mTOR and cAMP-related pathways have already completed clinical trials. In particular, the use of the vasopressin V2 receptor Tolvaptan has led to significant improvements in renal function[43], although treatment with mTOR signaling pathway inhibitors did not yield satisfactory results[44]. Investigation into the calcium modulator molecules as an alternative treatment for ADPKD has also begun. To this end, significant results have already been achieved in preclinical trials of triptolide, an active diterpene that induces intracellular calcium release through a PC2-dependent mechanism. Treatment with this molecule improved renal function in a mouse model for ADPKD, inhibiting cyst expansion by restoring normal calcium signaling and cell proliferation[45,46]. Conversely, retrospective studies have shown that treating ADPKD patients with calcium channel blockers provokes a worsening of renal function, as compared to untreated patients, by reducing the glomerular filtration rate[47]. However, treatment of PKD2(-/WS25) ADPKD mice with R-568, a type-2 calcimimetic molecule that triggers the activation of calcium-sensing receptors, showed no detectable effect on cystogenesis[48]. Nonetheless, despite the unsatisfactory results yielded by current therapeutic interventions relying on calcium channel modulators, it is worthwhile continuing this line of research, as further studies into other calcium regulators may lead to the discovery of more efficient drugs.

CONCLUSION

Polycystin complex, formed by the interaction between PC1 and PC2, may function as a calcium-permeable receptor-channel complex able to regulate intracellular calcium signaling. As both PC1 and PC2 are mutated in ADPKD, and in light of their effects on cell proliferation and apoptosis, considered typical hallmarks of ADPKD, it is highly plausible that such mutations play a central role in the disease. In complex or alone, PC1 and PC2 can both act in different cellular compartments, including the plasma membrane, endoplasmic reticulum and primary cilium, but the downstream effects of their dysfunction in ADPKD have still not been clarified. Nevertheless, it is known that functional loss of either PC1 or PC2 causes calcium signaling disruption, which is considered a primary event for kidney cyst formation in ADPKD. Although intracellular Ca^{2+} alteration abnormally activates several pathways that stimulate cell proliferation in ADPKD cystic cells, including cAMP-dependent B-Raf/MEK/ERK signaling and mTOR, EGFR and NFAT pathways, these are not activated in the cyst formation process associated with ciliary signaling impairment. Further investigation is therefore required to clarify the function of polycystins, and in turn identify new targets for ADPKD treatment.

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MINIREVIEWS

Percutaneous nephrolithotomy in pediatric age group: Assessment of effectiveness and complications

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Abstract

Management of kidney stone disease in pediatric population is a challenging condition in urology practice. While the incidence of kidney stone is increasing in those group, technological innovations have conrtibuted to the development of minimally invasive treatment of urinary stone disease such as mini-percutenous nephrolitotomy (mini-PCNL), micro-PCNL, ultra mini-PCNL. In this review we tried to evaluate the effect of new teratment techniques on pediatric kidney stones.

Key words: Percutaneous nephrolithotomy; Pediatric; Kidney stone; Urolithiasis

Core tip: In this article, minimally invasive treatment options of pediatric kidney stone disease are examined. Also, the effectiveness and complication rates of these techniques were reviewed in the light of recent publications.

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INTRODUCTION

The incidence of kidney stones in pediatric population is increasing and is reported that 50 cases per 100000 children[1]. The majority of kidney stones contain calcium. Most consist of calcium-oxalate but to a lesser extent calcium phosphate. Much less commonly kidney stones consist of urate, cysteine or struvite. Unlike adults, urinary stone disease in pediatric population is associated with genetic, metabolic and anatomical causes. Children with urolithiasis are considered high

risk for recurrent stone formation, and it is crucial for children to receive a treatment method that will provide them stone free[2].

Most pediatric urinary stones can be managed effectively by minimaly invasive treatment modalities such as extracoporeal shock wave lithotripsy (SWL), percutaneous nephrolithotomy (PCNL), retrograde intrarenal surgery (RIRS)[3]. However, PCNL can have a significant role in cases involving large and/or SWL resistant stones. Acording to the European Association of Urology guidelines, PCNL is recommended as primary treatment option for large renal stones (> 20 mm) and also for > 10 mm stones of the lower renal pole[4].

The surgical management of pediatric kidney stones with PCNL has been developed due to improvement of endoruologic devices and acquired experiences. Standard PCNL required 24-30 F nephrostomy sheath for renal access. But this method is associated with complications such as hemoglobin drop, blood transfusion, damage of renal parenchyma, and postoperative analgesic requirement. In order to decrease morbidity associated with PCNL in pediatric patients small size instruments have been used. Thus, PCNL is performed with small size endoscopes *via* smaller percutaneous tract in diameters ranging from 11 F to 20 F and this was named as Miniperc or Mini-PCNL[5]. Recently, Micro-PCNL or microperc has been described as another minimally invazive PCNL technique that is performed through a 4.8 F all-seeing needle[6].

The literature was reviewed for success and complication rates regarding recent PCNL techniques in pediatric age group.

MINI-PCNL: SURGICAL TECHNİQUE, SUCCESS AND COMPLICATION RATES

The first pediatric PCNL was described using a 15 F peel-away sheat and 10 F pediatric cystoscope by Helal *et al*[7] in 1977. Yet, this technique was developed using an 11 F access sheat by Jackman *et al*[8] in pediaric patients. Since then, the new form of PCNL has become a treatment option for adults as well[9,10]. The first 12 F nephroscope was presented to perform mini-PCNL in 2001[9]. The new device consisted of 15 F and 18 F sheats, a system of continuous low pressure irrigation, and a 6 F working channel. In time, this technique has developed and also accumulated in the pediatric patients for the treatment of renal stones regardless of the size of the stone. There is no common consensus as to exact size that is used for mini-PCNL, but usually access sheats below 20 F is accepted[11].

Mini-PCNL is performed under general anesthesia. After introduction of anethesia with the patient in the lithotomy position, retrograde ureteral catheterization is performed with 3-5 F ureteral catheter to fill the collecting system during percutaneous access. Then, the patient is repositioned in the prone posistion with a 30°-45° upward tilt of the affected site. Adequate padding of the pressure points should be done to prevent pressure induced injuries and neuropraxias[12,13]. Prone position is the most preferred technique but it has been reported that supine position *vs* prone position has equal safety and effectiveness[14]. Percutaneous renal access is achieved under the fluoroscopic and/or ultrasonic guidance. A lower pole posterior calyx access is preferred, but site of renal puncture may vary depending on localization and burden of stone and renal anatomy. Puncture tract dilatation is performed with dilators, followed by placement of the sheath. According to the endsocopic equipment used in mini-PCNL different sheath size has been reported in literature. Although most preferred one is 16 F sheath, 15 F, 16 F, 18 F or 20 F sheaths have been used. Also, the most common endoscopes used are 9 F, 5 F ureteroscope, 12 F and 15 F mini-nephroscopes[15,16]. Acording to the localization of the stone 7 F, 9 F and 14 F flexible ureteroscopes can be used. Stone disintegration is usually performed with laser and/or pneumotic lithotripsy that vary according to the surgeon preference[17].

PCNL is a challenging procedure in pediatric population because of the small kidney and the low tolerance to blood loss. The use of the mini-PCNL technique is becomig increasinly popular in the teratment of kindey stones in pediatric patients.

In the first publications, standart PCNL technique was performed for the treatment of kidney stone in children and stone-free rate (SFR) has been reported to be 47%-98%[18,19]. Adult instruments were used with minimal complications. Badway *et al*[19] reported their results of 60 children using a 26 F and 28 F Amplatz sheat. SFR was reported approximately 84% with PCNL monotherapy, with only one procedure being abandoned due to intraoperative bleeding. Samad *et al*[18] performed 188 PCNLs using a 17 F or 26 F nephroscope in children aged 6-16 years. SFR was reported 47% after PCNL momotherapy and transfusion rate was 3%. Bilen *et al*[20] compared the use of 26 F, 20 F and 14 F Mini-PCNL. The mean patient age of the children in each group was 13.2 years, 5.9 years and 6.3 years, respectively. The stone burden, previous surgery and the mean haemoglobin drop postoperatively did not change between the groups; however, the blood transfusion rate was higher in the 26 F and 20 F Amplatz sheath groups. The SFR was highest in the Mini-PCNL group, at 90%, compared to 69.5% in the 26 F and 80% in the 20 F group.

There is no consensus on definition of SFR. It is usually considered as stone fragments smaller than 3 or 4 mm. But untreated residual fragments can cause a stone related events. Due to the fact that pediatric patients have a risk for stone recurrence. It is important to achieve complete stone clearance by selected treatment methods in the treatment of kidney stones in pediatrics[21].

Wang *et al*[22] reported their results of 247 renal units with calculi in 234 patients who underwent mini-PCNL aged under 3 years. All procedure were performed by

Table 1 Mini- percutenous nephrolitotomy

Ref.	Year	Renal unit	Mean age	Stone size (mean)	Tract	Mean operative time (min)	Initial SFR %	Complications (%, overall)
Ozden *et al*[24]	2010	100	9.5 yr	507.5 mm²	20.8 F (mean)	79.1	85	25
Zeng *et al*[25]	2012	20	20.6 mo	2.2 cm	14-16 F	77.5	95	NR
Resorlu *et al*[26]	2012	106	9.6 yr	23.7 mm	12-22 F	76.3	85.8	17
Yan *et al*[27]	2012	27	42.6 mo	1.85 cm	14-16 F	86.5	85.2	15
Wah *et al*[28]	2013	23	4.76 yr	3.44 cm²	16 F	109.4	83.6	14
Onal *et al*[29]	2013	1205	8.8 yr	4.09 cm²	Cutoff size 20 F	93.5	81.6	27.7
Elderwy *et al*[30]	2014	47	8 (median) yr	2.3 cm (median)	20-24 F	90	91.4	10.6
Desoky *et al*[31]	2015	22	9.5 yr	2.4 cm	20 F	65.1	90.9	36.3
Brodie *et al*[15]	2015	46	7.3 yr	NM	16 F	NR	76	NR

NR: Non reported; NM: Not measured; SFR: Stone-free rate.

Table 2 Modified clavien classification

Grade Ⅰ	Any deviation from the normal postoperative course without the need for treatment
Grade Ⅱ	Requiring pharmacological treatment with drugs Blood transfusions and total parenteral nutrition are also included
Grade Ⅲ	Requiring surgical, endoscopic or radiological intervention
Grade Ⅲa	Intervention not under general anesthesia
Grade Ⅲb	Intervention under general anesthesia
Grade Ⅳ	Life-threatening complication requiring IC/ICU management
Grade Ⅳa	Single organ dysfunction (including dialysis)
Grade Ⅳb	Multiorgan dysfunction
Grade Ⅴ	Death of a patient

ICU: Intensive care unit.

single tract, including 245 14 F tracts, 1 16 F tract and 1 12 F tract, respectively. 191 cases had stone burden 1-2 cm² and 30 cases stone burden > 2 cm², 26 cases < 1 cm². Mean operating time was 32.5 min (range 21-62 min). Complete stone free rate has been reported as 240 renal unit (97.2%). In another mini-PCNL study SFR rates has been reported as 90.8% in stone burden < 20 mm, but 76.3% in stone burden > 20 mm[23]. In Table 1, there is an overwiew of the recent published data of mini-PCNL.

Due to the minimally invasive nature of mini-PCNL in the case of providing complete stone clearance and a clear nephrostomy tract makes the procedure in tubeless manner. Bilen *et al*[32] evaluated result of tubeless (ureteral catheter but no nephrostomy drainage tube) *vs* conventinal mini-PCNL (nephrostomy drainage tube) in infants and preschool children. In this study with 28 renal unit in 26 patients, the tubeles mini-PCNL group had significantly shorter surgery and fluoroscopy times. Complications rates were higher and duration of hospitalization were longer in the nephrostomy group. Stone-free rates were reported as 91.6% and 78.5% in tubeless and nephrostomy group, respectively.

The aim of the minimally invasive PCNL is to reduce complications such as blood loss, intraoperative -postoperative pain and hospital stay. On the other hand it is believed that a smal calibre tract is less injurious to nephrons. But many authors have reported that 24-26 F dilataion does not cause significant morbidity in children, it has been reported that there is no advantage in using a small access based on renal scaring alone[33]. The caliber and number of tracts are associated with intraoperative hemorrhage during PCNL in children[34]. Complication rates have significantly reduced with the development of the smallest and least traumatic endoscopic appliances. Moreover, it is reported that there is a significant correlation of intraoperative bleeding with duration of surgery, stone burden and sheath size[35]. In additon that it is stated that operative time, sheat size, mid calyceal puncture and partial staghorn formation are independet predictors of complications[29].

It is important that using a common definition in the expression of complication to determine the risk factors for complications. Recently, the modified Clavien system for classifying surgical complications has been used for this purpose[36]. But complications are not always reported according to this system in recent publications (Table 2). Modified Clavien Classification has been shown.

The fist time, Ozden *et al*[24] indicated perioperative complications of PCNL in pediatric patients using the modified Clavien grading system. Transient fever (grade I) is one of the most frequent complication. But it is not always microbial in origin[37]. It is determined that transient fever rate is 31% in 188 PCNLs. However, postoperative infection is reported in approximately 6% of pediatric patients[20,38].

Bleeding is a serious complication during intraoperative and postoperative period in pediatric patients which is associated with sheath size, stone burden, number of tracts and operative time. Hemoglobin drop requiring transfusion (gare Ⅱ) is reported in 0.4%-24% of patients[39,40]. In another study higher hemoglobin drop

Table 3 Complication rates of mini- percutenous nephrolitotomy accoring to modified clavien classification

Ref.	Year	Renal unit	Overall complication rate (%)	Grade Ⅰ-Ⅱ (%)	Grade Ⅲ (%)	Grade Ⅳ-Ⅴ (%)
Ozden *et al*[24]	2010	100	25	21	4	-
Resorlu *et al*[26]	2012	106	17	17	-	-
Yan *et al*[27]	2012	27	15	15	-	-
Wah *et al*[28]	2013	23	14	13.6	0.4	-
Onal *et al*[29]	2013	1205	27.7	23.04	3.46	1.2
Pan *et al*[42]	2013	59	11.9	11.9	-	-
Elderwy *et al*[30]	2014	47	10.6	8.5	2.1	-
Desoky *et al*[31]	2015	22	36.3	22.7	13.6	

has been determined in pediatric patients performed PCNL when size of the tract dilataion exceeded 22 F[34].

There is a debate on the classification of grade Ⅲ complication, is that auxillary procedures such as RIRS, SWL and second look PCNL. It is recommended to considered them as part of treatment strategy. However, such as hydrothorax requiring chest tube or urine leakage requiring urinary diversion can be classified as Clavien grade Ⅲ complication[24]. It is said that grade Ⅲ, Ⅳ, Ⅴ complications should be quite rare and more likely associated with surgical techniques and expereince[41]. Complication rates have been shown in literature in Table 3.

ULTRA-MINI PCNL: SURGICAL TECHNIQUE AND NEW REPORTS

In 2013, the new PCNL technique was described by Desai *et al*[34] using of a novel 6 F mini nephroscope through an 11-13 F metal sheath to perform holmium: YAG laser lithotripsy. The new procedure was performed in 36 patients with a mean stone size 14.9 mm. Two patient were preschool children. It was reported that mean operative time, stone free rate at postoperative 1st day and 1st month were 59.8%, 88.9%, and 97.2%, respectively. Complication rate were reported as 16.% in 6 patients, according to Clavien classification, including 2 sepsis, 1 urinary extravasation, and 3 fever. The authors determined that there was no needed blood transfusion[43]. In another study results of 62 patients were reported using a 3.5 F nephroscope. Nephrostomy tract was dilatated up to 13 F. Only four of the 62 patients were children. Mean stone size was 16.8 mm, stone free rate at the 1st month was reported approximately 87%[44]. There is no sufficient data available to compare this new technique with other methods which use for the treatment of pediatric urinary stones. The new technique's effectiviness and safety remain to be seeen in larger prospective studies in pediatric patients.

MICRO-PCNL: SRUGICAL TECHNIQUES

Recently, Micro-PCNL or microperc has been described as another minimally invasive PCNL technique that is performed through a 4.85 F all-seeing needle. A three-way connector is attached to the latter, which admits a saline irrigation tube, 0.9 or a 0.6 mm-diameter micro-optic, a 272 μm laser fiber. The outher diameter of this modified needle is 1.6 mm (4.85 F). The first time this new technique were used in 15 adults. Mean stone size, operation time was 30.4 mm, 101.4 min, respectively. Postoperative complete stone clearance achieved in 11 patients[45]. Since then this method has addopted to the tretamnet of pediatric kidney stones. In a study, 24 infant treated with micro-PCNL. The mean age, stone size, operation time were 15.8 mo, 13.5 mm, 53.7 min, respectively. There is no major complication and hemoglobine drop requiring blood transfusion reported[46]. More experience and more knowledge is needed for the effectiveness of this method.

CONCLUSION

Technological innovations have contributed to the development of minimally invasive treatment of urinary stone disease. It can be said that to increase the efficacy and reduce complications is the main objective of physcians. In this manner new teratment methods which use for minimally invasive management of kindey stones in pediatric population has offered various treatment alternatives to the surgens. However, level of experience and new publications can contribte us to provide complete stone clearance and to reduce complication rates.

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Central blood pressure and chronic kidney disease

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Abstract

In this review, we focused on the relationship between central blood pressure and chronic kidney diseases (CKD). Wave reflection is a major mechanism that determines central blood pressure in patients with CKD. Recent medical technology advances have enabled non-invasive central blood pressure measurements. Clinical trials have demonstrated that compared with brachial blood pressure, central blood pressure is a stronger risk factor for cardiovascular (CV) and renal diseases. CKD is characterized by a diminished renal autoregulatory ability, an augmented direct transmission of systemic blood pressure to glomeruli, and an increase in proteinuria. Any elevation in central blood pressure accelerates CKD progression. In the kidney, interstitial inflammation induces oxidative stress to handle proteinuria. Oxidative stress facilitates atherogenesis, increases arterial stiffness and central blood pressure, and worsens the CV prognosis in patients with CKD. A vicious cycle exists between CKD and central blood pressure. To stop this cycle, vasodilator antihypertensive drugs and statins can reduce central blood pressure and oxidative stress. Even in early-stage CKD, mineral and bone disorders (MBD) may develop. MBD promotes oxidative stress, arteriosclerosis, and elevated central blood pressure in patients with CKD. Early intervention or prevention seems necessary to maintain vascular health in patients with CKD.

Key words: Atherosclerosis; Mineral and bone disorder; Oxidative stress; Proteinuria; Renal autoregulation

Core tip: Wave reflection is a major mechanism that determines central blood pressure in chronic kidney disease (CKD). Diminished renal autoregulatory ability characterizes CKD, allowing an increase in proteinuria. Thus, any elevations of central blood pressure accelerate the progression of CKD. The kidney produces oxidative stress compounds due to proteinuria handling and secondary interstitial inflammation. Oxidative stress facilitates atherogenesis, increases arterial stiffness and central blood pressure. Furthermore, even in early stages of CKD, mineral and bone disorder (MBD) is

developed. CKD-MBD facilitates to induce oxidative stress and elevation of central blood pressure. To keep vascular health in CKD, early intervention or prevention seems mandatory.

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INTRODUCTION

Blood pressure is the product of the cardiac output and total peripheral vascular resistance. In turn, cardiac output is the product of the stroke volume and heart rate. The diastolic and mean blood pressures remain similar along the systemic arterial tree[1]. Therefore, the aortic and brachial mean blood pressures are comparable[1]. However, systolic blood pressure differs significantly between the central and peripheral arteries, even within a single cardiac beat. Specifically, the central systolic blood pressure is lower than the brachial systolic blood pressure, which itself is lower than the systolic blood pressure at the dorsal foot artery. At any given site within the arterial tree, the systolic blood pressure increases as the distance from the heart increases[2].

CENTRAL HEMODYNAMIC MECHANISMS

How does a single heart stroke cause variations in blood pressure from the aorta to the peripheral arteries? Two mechanisms have been proposed (Figure 1): Wave reflection and amplification[2]. Wave reflection occurs at all levels of the arterial tree[3]. Reflection occurs in areas where the arterial caliber is decreasing, or at areas where a single artery divides into two or three branches. Each wave reflection causes a backward wave in arterial system. If all backward waves could be integrated, the single wave would ascend approximately from the aortic bifurcation. Backward and forward waves yield summation effects, resulting in augmented systolic blood pressure. As shown in Figure 2, the augmentation index (AI) is defined as the augmented pressure/forwarding pulse pressure. The summation effects are affected by many factors[4], including the degree of wave reflection, heart rate, height, and pulse wave velocity (PWV). The reflection magnitude is modulated by the stroke volume and arterial stiffness. A greater stroke volume enlarges the reflection. Increased arterial stiffness, such as that in elderly individuals, also increases reflection. A short height and fast PWV also allow the backward wave to reach the ascending aorta during systole, resulting in a very high central systolic blood pressure and significant stress on the left ventricle[5,6]. Normally, the backward wave arrives at the ascending aorta in diastole, facilitating coronary perfusion. A slower heart rate lengthens the ejection period and allows the backward wave to reach the ascending aorta at late systole. Thus, the early arrival of a large reflection wave at the ascending aorta increases both the central systolic blood pressure and cardiovascular (CV) risk.

Pressure amplification is a physiological phenomenon that is evident in young people with supple, flexible, elastic arteries[2]. In such individuals, the arterial wall flexes like a whip with each heart stroke. We will attempt the difficult process of explaining pressure amplification without a mathematical analysis. During the systolic phase, the pulse wave arrives at the aorta and proceeds at the speed of the PWV. The PWV speed is well known to increase along with systolic blood pressure. One can divide the forwarding pulse into three parts: Initial, middle, and last. In the initial part, blood travels from the heart to the aorta, which is very soft and becomes distended. Accordingly, the PWV is slow in the initial part and functionally increases the aortic root stiffness with a small increase in blood pressure. During the middle part, the forwarding pulse emerges from the heart. As the aortic root stiffness is higher in the middle part than in the initial part, the PWV also increases. Consequently, some of the middle part catches up with the initial part, leading to a moderate amplification of systolic pressure. Finally, the last part enters the aorta at the fastest PWV, further amplifying the systolic blood pressure. Closure of the aortic valve ends this escalation of systolic pressure. Importantly, this pressure amplification continues along the length of the aorta as the pulse wave travels. Thus, in young people, the central systolic blood pressure remains low, compared with the brachial systolic blood pressure[7].

Indeed, isolated systolic hypertension is a CV risk in elderly patients, not in young subjects[7]. The above description may explain why pressure amplification is a major cause of isolated systolic hypertension in young individuals, whereas wave reflection causes central systolic blood pressure elevation in the elderly. The Framingham Study focused attention toward pulse pressure as the best measure of CV risk, at least in older subjects[7]. Since pulse pressure is a surrogate measure of arterial stiffness, such data indicate that arterial stiffness is a key determinant of CV risk in older subjects. Although there is a debate, the data from Framingham study suggest that diastolic pressure remains the best predictor of coronary heart disease risk in younger subjects. As chronic kidney disease (CKD) is rather common in elderly populations whose artery is stiff due to the remodeling[2,5,6], wave reflection, rather than pressure amplification, determines the central blood pressure in this patient population.

CENTRAL BLOOD PRESSURE MEASUREMENT METHODS

The central systolic blood pressure places a direct

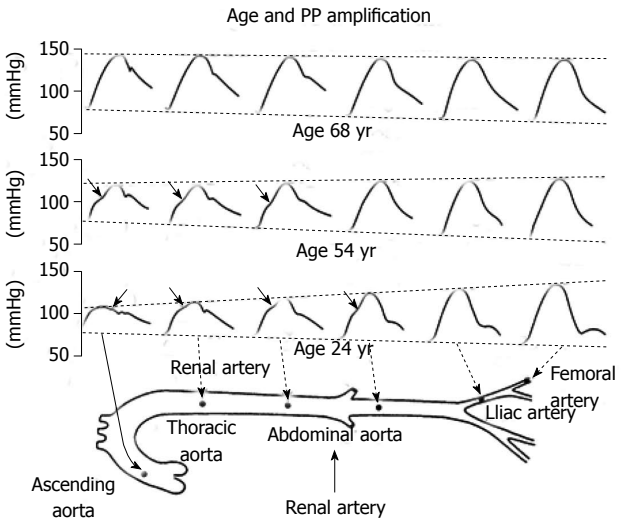

Figure 1 Representative pulse waveforms along the aorta in young, middle-aged and elderly persons. In younger subjects (age: 24 yr), the rate of propagation is relatively low in arterial vessels, which become progressively narrower and less distensible. Because of the summation of the forward and the backward wave at each point of the arterial tree, peak systolic blood pressure increases markedly from central to peripheral arteries, while end-diastolic blood pressure tends to be reduced and mean arterial pressureremains unchanged. In older subjects (age: 68 yr), because of the more rapid propagation of pressure wave with resulting changes in wave reflections, the amplification of PP disappears, making that central and peripheral BP become identical. At 54 yr of age, the situation is intermediate between younger and older subjects[2].

burden on the left ventricle and is a better predictor of CV prognosis than the brachial blood pressure. Central blood pressure correlates better with real blood pressure for heart and great vessels than brachial blood pressure. A lower central blood pressure is associated with a better CV outcome, regardless of brachial blood pressure. Until recently, intravascular catheterization was only the method to measure central blood pressure efficiently. This method is direct and accurate, and therefore remains the gold standard for central blood pressure assessment. However, it is so invasive that only selected patients can undergo such an evaluation. Recent progress in medical technologies has enabled non-invasive assessments of central blood pressure.

Currently, two devices that provide consistent central blood pressure readings are available on the market[8]. First, Karamanoglu *et al*[9] performed invasive simultaneous measurements of both the brachial and aortic pulse waveforms and used a Fourier analysis to generate a generalized transfer function. This transfer function allows the estimation of an aortic waveform from a brachial waveform. The transfer function was later used to develop a device that uses a tonometer to access the radial pulse waveform and estimate an aortic pulse waveform (Figure 3). This device is able to calculate the aortic blood pressure through calibration with indirect brachial blood pressure measurements obtained *via* the cuff method. Second, Takazawa *et al*[10] independently developed a new device to access the central blood pressure. The authors invasively measured the aortic blood pressure during cardiac catheterization, while simultaneously indirectly measuring both the radial pulse waveform and brachial blood pressure. They found that the second peak of radial pulse waveform correlated with the aortic waveform peak, thus enabling an indirect estimation of the aortic systolic blood pressure without using the generalized transfer function (Figure 4).

Notably, cuff measurements of brachial blood pressure *via* oscillometric methods have such large errors that invasive measurements of the brachial blood pressure are approximately 10 mmHg higher than non-invasive measurements[8]. Both devices have been described as calibrating the central blood pressure through the indirect measurement of brachial blood pressure. Thus, invasive measurement yields central blood pressure values approximately 10 mmHg higher than device-assisted indirect central blood pressure values. Although we are very familiar with the indirect measurement of brachial blood pressure, great cautions are required when discussing the accuracy of the method to assess the exact blood pressure.

INCREASED CENTRAL BLOOD PRESSURE IS AN IMPORTANT CV RISK

Recent clinical studies have shown that the increase in central blood pressure is a stronger CV risk than the brachial blood pressure. Williams *et al*[11] divided a cohort of enrolled hypertensive Anglo-Saxon and Scandinavian patients into two groups: Those treated with calcium channel blocker-based medications, and treated with beta-blocker-based regimens. During the follow-up period, both groups exhibited similar brachial blood pressure control. However, fewer CV events occurred in patients receiving calcium channel blocker-based therapy. Importantly, the central blood pressure was significantly lower in those treated with calcium channel blockers than in those treated with beta-blockers (Figure 5). The authors also demonstrated that central blood pressure contributed to the number of total CV events and the development of renal impairment, suggesting that a correct central blood pressure measurement is a more accurate parameter than brachial blood pressure in preventing CV and renal events. Roman *et al*[12] performed a population-based longitudinal study of prevalent and incident CV disease in 3502 American Indians; 319 of these subjects suffered fatal and non-fatal CV events during a 5-year follow-up. The authors concluded that the measurement of central blood pressure more strongly predicts CV events than does brachial blood pressure. However, Chirinos *et al*[13] enrolled 2606 patients with CKD patients and observed the incidence of hospitalization for new-onset heart failure over a 3.5-year period. These authors concluded that a fast aortic PWV, but not a high central blood pressure, predicted heart failure. It is difficult to distinguish heart failure from fluid retention in patients with CKD partly due to vascular remodeling including calcification. In contrast, our previous study indicated

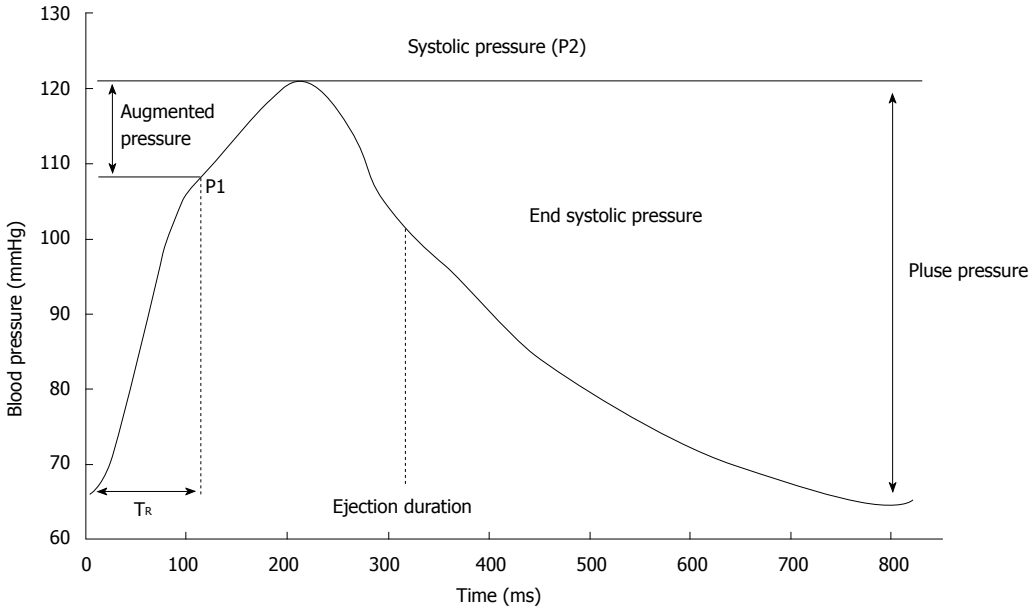

Figure 2 Example central pressure waveform. TR indicates timing of the reflected pressure wave; P1 and P2 represent the first and second systolic peaks, respectively[3]. Augmentation index is defined as augmented pressure/forwarding pulse pressure (P1).

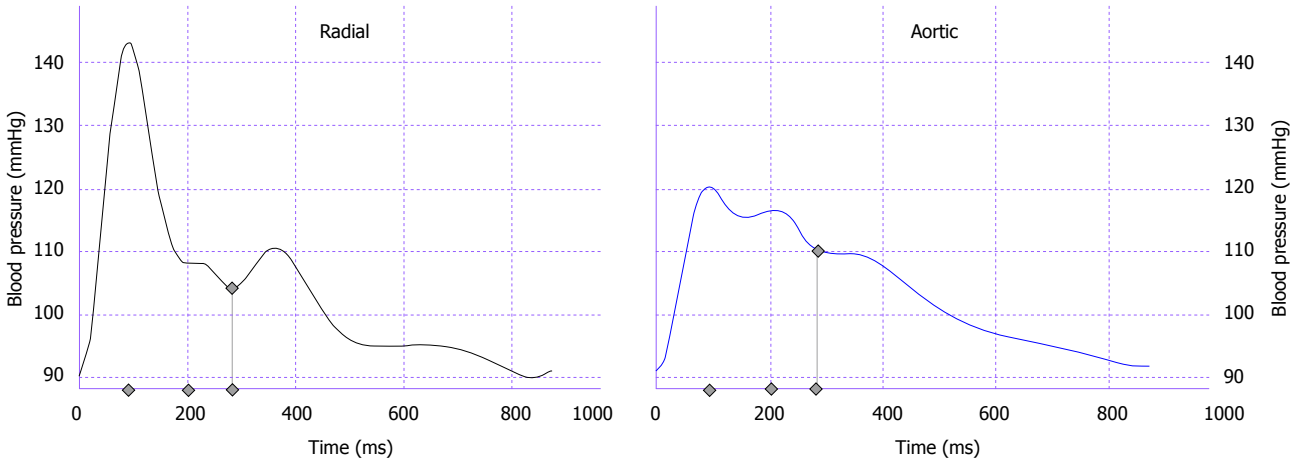

Figure 3 Estimation of central pulse waveform. Radial tonometry detects radial pulse waveform with high systolic blood pressure over 140 mmHg (left panel). Using this radial waveform, generalized transfer function calculates aortic pulse waveform (right panel). Please note that aortic systolic blood pressure is 120 mmHg (Available from: URL: http://hogimed.fr/?q=sphyg%20p1).

that AI predicted CV events in hemodialysis patients[14]. Collectively, these data suggest that the blood pressure in the ascending aorta is a significant CV risk for the development of atherosclerotic CV diseases.

What about abdominal aortic blood pressure, to which the kidney is exposed? The backward wave travels for a shorter distance and meets the forwarding wave sooner in the aorta at the renal artery level, compared to the ascending aorta. Thus, the backward wave augments the forwarding wave in mid-systole, leading to greater summation effects[5]. The pressure amplification at this level is also greater than in the ascending aorta because the forwarding wave travels a longer distance. Collectively, the renal arterial pressure should fall between the aortic root and brachial blood pressures. Indeed, Hope *et al*[15] examined blood pressure profiles along the aorta during cardiac catheterization in patients with an average age of 65 years, and reported that the aortic systolic pressure was approximately 10 mmHg higher at the level of the kidney than in the ascending aorta.

CENTRAL BLOOD PRESSURE AS A CAUSE OF CKD

When exposed to high blood pressure, the arteries and arterioles constrict and increase their vascular resistance to buffer the direct transmission of systemic pressure to capillary beds in the terminal organs, in a process called autoregulatory or myogenic vasoconstriction[16]. In addition to myogenic constriction, tubuloglomerular feedback (TGF) affects renal autoregulation[17]. TGF

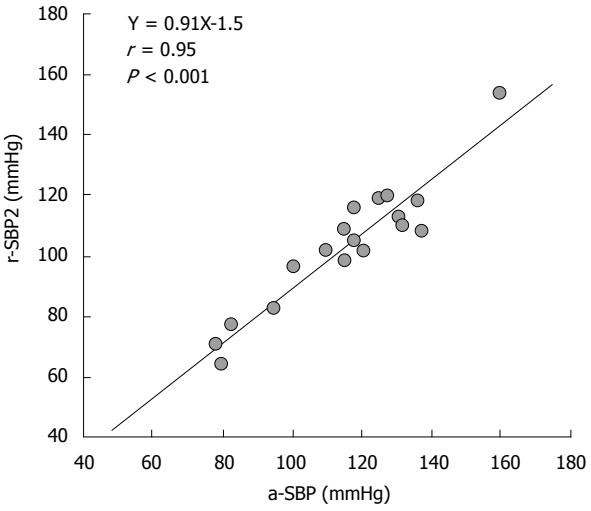

Figure 4 Relationship between radial second peak of systolic blood pressure and aortic systolic blood pressure. There is a strong positive relation between two[10]. r-SBP2: Radial second peak of systolic blood pressure; a-SBP: Aortic systolic blood pressure.

is the mechanism specific for the kidney to maintain glomerular filtration rate constant. Elevations of blood pressure temporally increase both glomerular capillary pressure and filtration rate. This elicits an increase in tubular flow that reaches macula densa. Then, the reabsorption by macula densa is increased. Macula densa cells release the mediator to constrict afferent arteriole, thereby returning both glomerular capillary pressure and filtration rate to the baseline. Many studies have repeatedly demonstrated that the dysregulation of these autoregulatory responses in CKD[18]. Nephritic patients commonly exhibit mesangial changes, which damage the effectiveness of TGF[19]. In nephrosclerosis, afferent arteriolar changes such as hyalinosis (benign nephrosclerosis) and fibrinoid necrosis (malignant nephrosclerosis) preclude the normal autoregulatory behavior of the afferent arteriole[20]. In diabetes, hyperglycemia facilitates the re-uptake of NaCl through sodium-glucose co-transporters at the proximal tubules. NaCl delivery to the macula densa is reduced in diabetes, thereby reducing the TGF[21]. CKD is characterized by the diminished TGF, activating renin-angiotensin system, causing secondary hyperaldosteronism, volume expansion and hypertension. Consequently, in patients with CKD, systemic blood pressure is transmitted rather directly to the glomeruli, partly because of inadequate renal autoregulatory adjustments. Thus, glomerular hyperfiltration and hypertension are commonly observed in this patient population.

Proteinuria is a clinical marker of glomerular hypertension[21]. We performed clinical studies to determine the role of central hemodynamics in CKD progression. As the aortic blood pressures at the renal artery and aortic root differ, we focused on central hemodynamic parameters such as AI and the time for reflection (TR), rather than the central blood pressure itself. TR indicates the time required for the reflection pressure to arrive at the ascending aorta (Figure 2). As discussed above, an inappropriate activation of renin angiotensin system is common in CKD. Our previous data indicated that AI correlated positively with proteinuria in 99 non-diabetic patients with CKD[5]. Among 44 patients with angiotensin inhibition, a higher basal AI led to a greater annual decrease in creatinine clearance (Figure 6), suggesting that in addition to angiotensin, AI is a risk factor for the progression of non-diabetic CKDs. We further performed an observational study of 42 non-diabetic patients with CKD[22]. A multivariate regression analysis revealed a correlation between annual increases in serum creatinine and the TR, suggesting that the TR predicts the progression of renal dysfunction in patients with CKD. Finally, we performed a randomized controlled trial of 59 hypertensive CKD patients to assess the long-term effects of calcium antagonists on AI[23]. All patients received an angiotensin receptor blocker and amlodipine or azelnidipine. Compared to amlodipine, azelnidipine reduced proteinuria and AI to a greater extent. Consequently, these data support the notion that any reductions in the abdominal aortic blood pressure would decrease proteinuria, thus slowing the progression of CKD.

Recent findings indicate an increase in blood pressure variability causes kidney damage[24]. Blood pressure exhibits beat-to-beat, day-to-day, and visit-to-visit variations. Renal autoregulatory adjustments occur with some delay following changes in blood pressure[16,17]. The myogenic mechanism requires a few second to initiate, and the TGF requires a slightly longer time to complete its final adjustment. If the blood pressure suddenly decreases, the low blood pressure must perfuse the kidney, which exhibits high vascular resistance due to the remaining autoregulatory vasoconstriction; this situation presumably leads to renal ischemia. If the blood pressure increases abruptly, this high systemic blood pressure is transmitted to the glomeruli rather directly before an adequate autoregulatory increase in renal vascular resistance can occur. Thus, marked blood pressure variability may induce ischemia-reperfusion type renal damage[25]. Of interest, a high aortic pulse pressure was found to correlate with an increase in beat-to-beat blood pressure variability[26]. Collectively, central hemodynamic abnormality hastens the progression of CKD presumably by causing renal ischemia in addition to glomerular hypertension.

INCREASED CENTRAL BLOOD PRESSURE AS A CONSEQUENCE OF CKD

The glomeruli continuously leak proteins into the ultrafiltrate[27]. These filtered proteins are largely taken up by proximal tubular cells and handled in one of two ways. Under physiological conditions, the glomerulus leaks a small amount of proteins that are absorbed by proximal tubular cells and subjected to acid hydrolysis. Under pathological conditions, such as proteinuric CKD, each

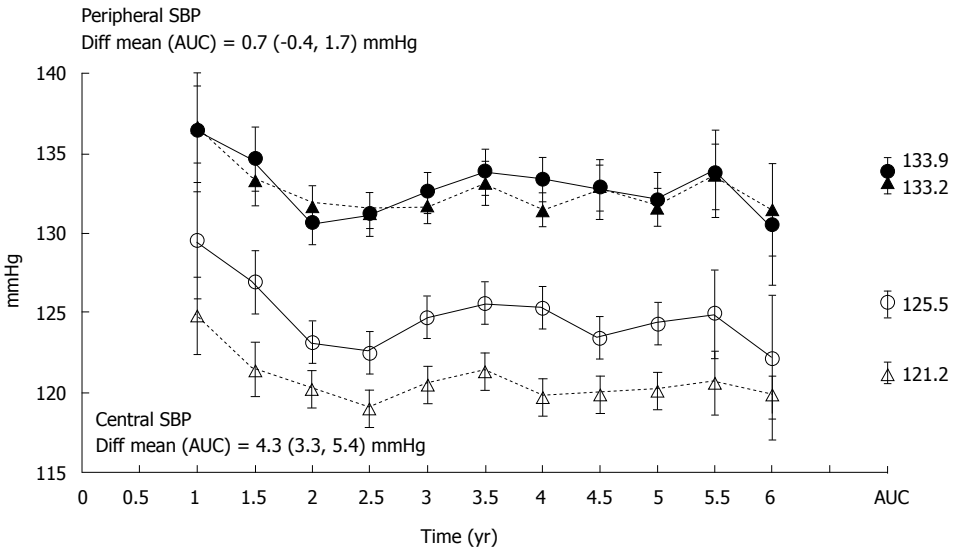

Figure 5 Principal results of ASCOT-CAFÉ study. Brachial blood pressure was similar between the patients treated with beta-blocker-based medication (close circles) and calcium channel blocker-based treatment (closed triangles). However, central blood pressure was higher in the former (open circles) than the latter group (open triangles)[11]. SBP: Systolic blood pressure.

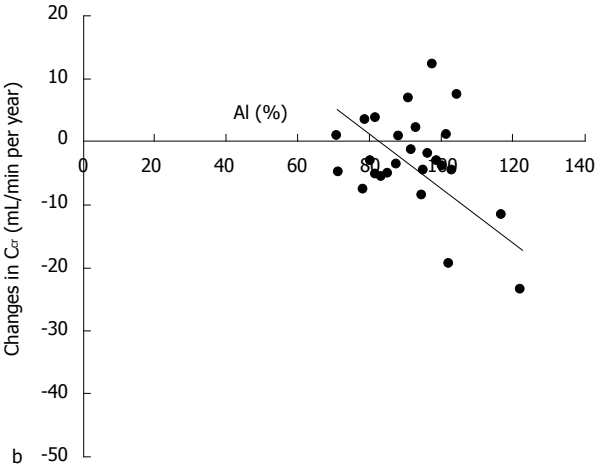

Figure 6 Relationship between annual changes in creatinine clearance and augmentation index. There is an inverse relation between two[5].

glomerulus leaks a large amount of proteins. Because the capacity of the proximal tubules to hydrolyze proteins is limited, oxidative degradation begins to break down the absorbed proteins, thus triggering an atherogenic chain reaction. Reactive oxidative species (ROS) diffuse into the peritubular capillary and oxidize circulating molecules such as low-density lipoprotein cholesterol (LDL-C). Oxidized LDL-C consequently induces inflammation in the arterial wall to initiate an atheroma[28]. Subsequently, these atheromas become focal points for *de novo* oxidative stress and promote progression to systemic atherosclerosis. In addition to generating oxidative stress, the proximal tubular cells secrete various chemokines that recruit inflammatory cells into the renal interstitium. In turn, interstitial inflammation accelerates oxidative stress. Non-proteinuric CKDs, such as hydronephrosis, cystic kidney disease, and ischemia-reperfusion, also increase oxidative stress[25]. Cystic expansion or increased intratubular pressure causes tubular cell damage and can induce apoptosis or necrosis. In a manner similar to that observed in proteinuric CKDs, inflammatory cells accumulate in the renal interstitium to remove the debris and replace it with fibrotic material. Epithelial-mesenchymal transition may contribute to fibrosis process. Interstitial inflammation also triggers an atherogenic chain reaction in non-proteinuric CKDs.

Atherosclerosis is characterized by arterial stiffness, for which PWV is a good index. The carotid-femoral (cf) PWV has been used in many studies. We previously conducted an observational study of 102 hypertensive patients with CKD[29]. These patients were divided into two groups according to the use or non-use of an angiotensin converting enzyme inhibitor or angiotensin receptor blocker and observed for 4 years. The heart-femoral (hf) PWV was measured repeatedly. Compared with cfPWV, which measures arterial stiffness below the aortic arch, hfPWV assesses arterial stiffness across the total aorta and iliac artery. Brachial blood pressure was similarly controlled in both groups. However, although gradual hfPWV elevation was observed in the group without angiotensin inhibition, this value remained unchanged in the patients under angiotensin inhibition. As expected from the lack of a progressive increase in PWV[30], angiotensin inhibition reduced both CV and renal deaths. In addition, PWV correlated positively with AI in patients with CKD[22]. In other words, a rapid PWV indicates a high AI and central blood pressure. Maintenance of the central blood pressure within a normal range is a mandatory step in maintaining renal blood flow and glomerular filtration without inducing oxidative stress. It would be advantageous to slow the progression of renal dysfunction in CKD. From these results, we propose a working hypothesis in which a vicious cycle exists between CKD and increased central

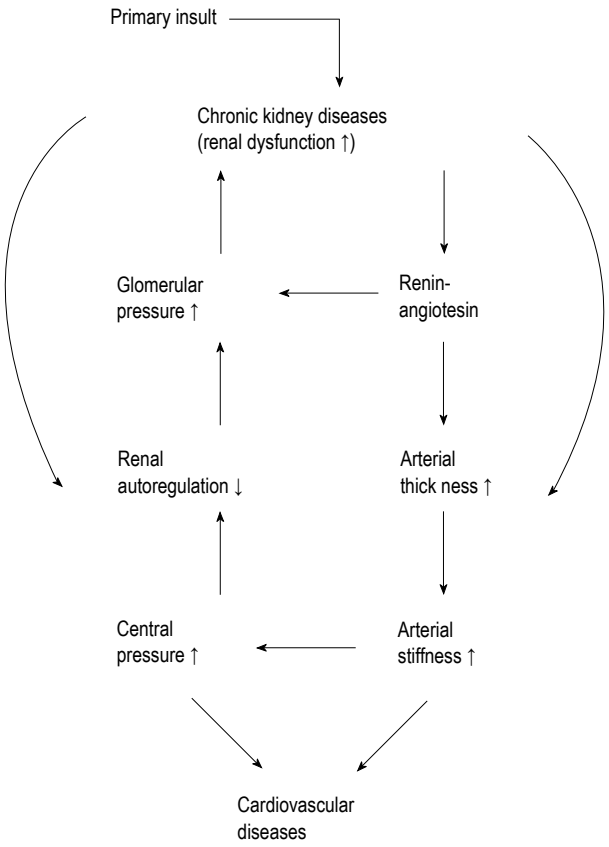

Figure 7 Working hypothesis underlying cardiovascular disease and chronic kidney disease. Lowering central blood pressure could cut vicious cycle between CVD and CKD[29]. CKD: Chronic kidney disease; CVD: Cardiovascular disease.

blood pressure (Figure 7).

Mineral and bone disorders (MBDs) also underlie the development of CV diseases in CKD[31]. Notably, MBDs are initiated at an early stage of CKD. For example, renal klotho expression is already reduced at CKD stage 2. This situation induces an increase in FGF23 expression. In CKD stage 3, a decreased calcitriol level and secondary hyperparathyroidism are common observations. In CKD stage 4-5, hyperphosphatemia and hypocalcemia become evident. FGF23 increases ROS production in vascular smooth muscle cells and induces cardiac hypertrophy[32,33]. In vascular smooth muscle cells, the excessive uptake of phosphate through Pit1 induces the expression of osteocyte-specific genes, thus changing the cellular phenotype from vascular to bone[34]. Arterial calcification is a significant CV risk, especially in patients with advanced CKD[35]. Aortic root calcification is common in this population. Lam *et al*[36] demonstrated that aortic root remodeling is a significant CV risk. We performed a cross-sectional study to characterize the central hemodynamics in 1392 CKD patients. As shown in Figure 8, the AI was lower in stage 5 than in stage 1[37]. Because of the marked increase in aortic root stiffness, the forwarding wave cannot adequately stretch the aortic root; subsequently, the forwarding pressure increases to the extent that the reflection pressure contributes slightly to the peak aortic pressure, thus reducing the AI. Collectively, our results provide functional evidence that aortic root stiffness is markedly increased in stage 5 CKD, which would account for the high CV risk faced by advanced CKD patients. In addition, our data indicated that diastolic blood pressures were lower in CKD stages 3-5 were lower than stage 1. Under physiological conditions, aorta stores approximately half of the stroke volume during systole. The pooled blood keeps the organ well perfused during diastole. Increased aortic stiffness not only decreases this storage capacity and thus reduces coronary perfusion, but also elicits central high blood pressure and resultant left ventricular hypertrophy[38,39]. Thus, increased aortic stiffness exacerbates myocardial ischemia, worsening CV prognosis.

POSSIBLE THERAPIES FOR CENTRAL HIGH BLOOD PRESSURE IN CKD

Most patients with CKD manifest hypertension, and the selection of antihypertensive agents might determine their central blood pressure[40]. Vasodilating antihypertensive agents, including calcium channel blockers, angiotensin receptor blockers, converting enzyme inhibitors, and alpha-adrenergic blockers, preferentially reduce the central blood pressure rather than the brachial blood pressure (Figure 9). Aliskiren was not available in the market when this study was performed. In contrast, non-vasodilating antihypertensive medications, such as diuretics and beta-adrenergic blockers, similarly reduce the central and brachial blood pressures. Thus, the administration of vasodilator antihypertensive agents to hypertensive patients with CKD more efficiently lowers the central blood pressure, compared with non-vasodilator antihypertensive medications, thereby ameliorating proteinuria and preventing the development of atherosclerotic CV diseases. In this regard, patients whose blood pressure had not reached goal values, despite treatment with an angiotensin receptor blocker, were evaluated in a retrospective study[41]. Patients treated with additional calcium channel blockers or additional diuretics were compared. Both calcium channel blocker and diuretic treatment considerably reduced the brachial blood pressure. However, although both agents reduced the AI, calcium channel blockers yielded greater improvements in this parameter. Compared with those using diuretics, patients using calcium channel blockers exhibited a greater decrease in protein excretion. Interestingly, decreases in proteinuria correlated with reductions in AI. Similarly, Bakris *et al*[42] demonstrated that combined treatment with converting enzyme inhibitors and calcium channel blockers provided better renal protection than combined treatment with both converting enzyme inhibitors and diuretics. Although angiotensin receptor blockers, converting enzyme inhibitors, and direct renin inhibitors have been established as first-line antihypertensive drugs for proteinuric patients with CKD[43], calcium channel

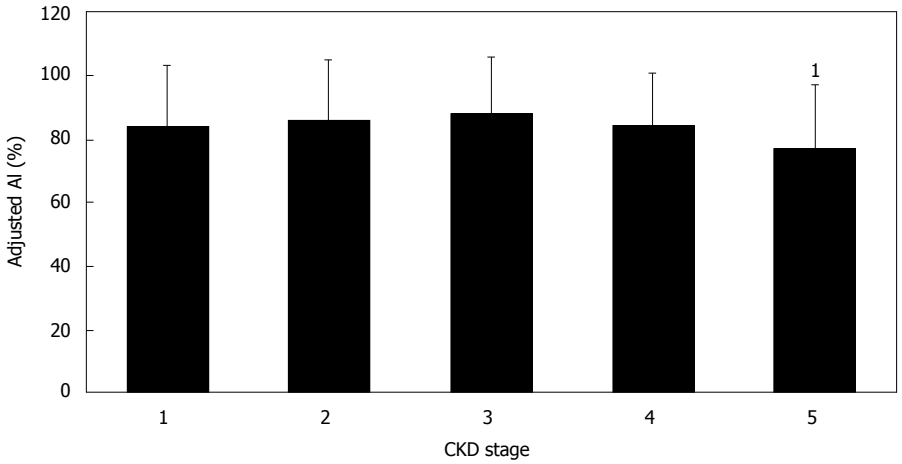

Figure 8 Comparison of augmentation index among all chronic kidney disease stages. AI was adjusted with confounding factors including age, blood pressure, pulse rate, and vasodilator antihypertensive drugs. [1]Indicated significant difference from stage 1[37]. CKD: Chronic kidney disease.

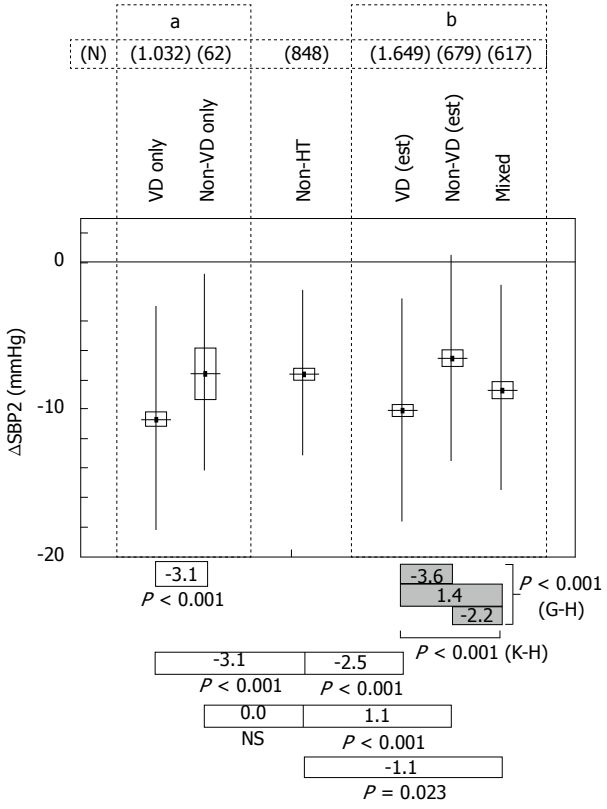

Figure 9 Disparate effects of antihypertensive drugs on central blood pressure. Differences between brachial systolic blood pressure and central systolic blood pressure (ΔSBP2) were compared between vasodilator (VD) and non-vasosilator (non-VD) antihypertensive medications. ΔSBP2 was adjusted by age, gender, height, BMI, diastolic blood pressure and use of nitrate. The comparison between actual VD and non-VD only regimen was shown in left panel (A). Right panel (B) depicted comparisons among VD(est), non-VD(est) and Mixed combination of VD and non-VD. "(est)" indicated including data derived from mixed combination, for which the effects of VD and non-VD alone on ΔSBP2 were estimated. Non-hypertensive population (non-HT) was used as physiological reference for ΔSBP2. VD antihypertensive drugs included angiotensin receptor blocker, calcium channel blocker, converting enzyme inhibitor and alpha-blocker. Non-VD group includes beta-blocker and diuretics. Mann-Whitney U test was used to compare the means, unless otherwise specified. K-W and G-H described Kruskal-Wallis and Games-Howell multiple comparison test[40].

blockers appears more suitable than diuretics for second-line antihypertensive treatment. In addition, calcium channel blockers flatten intra-individual variations in blood pressure[44]. Therefore, calcium channel blockers appear to retard the progression of hypertensive non-proteinuric CKD by preventing additional ischemia-reperfusion renal damage.

Endothelial cells secrete nitric oxide in response to shear stress, thus relaxing the arteries[45]. Alternatively, oxidative stress reduces the bioavailability of nitric oxide, which elicits vasoconstriction and arterial remodeling[46]. Clinically, flow-mediated vasodilation (FMD) can be used to assess endothelial function[47]. Blood flow stimulates the endothelium to release vasodilators such as nitric oxide, and the effects of the vasodilators can be assessed by monitoring arterial diameter with ultrasound device. We enrolled 36 CKD patients with dyslipidemia to evaluate the effects of statin on FMD[27]. Although FMD is was reduced in patients with CKD, this parameter correlated inversely with the magnitude of proteinuria. Furthermore, atorvastatin treatment improved both FMD, as well as LDL-C levels. In addition, our previous data suggest that combined treatment with statins and angiotensin inhibitors attenuated the progressive increases in PWV observed in hemodialysis patients[48]. Statins exert pleiotrophic actions, including immunomodulation, anti-inflammation, and oxidative stress reduction[49]. Statins also inhibit both podocyte injury and protein re-uptake by proximal tubules (Figure 10)[27]. As discussed, oxidative stress is a mediator of atherosclerosis development in CKD. In patients with CKD, the judicious use of statins might help to end the vicious cycle between the progression of renal dysfunction and central high blood pressure.

A recent study demonstrated that when compared with calcium carbonate treatment, sevelamer hydrochloride treatment for the control of hyperphosphatemia slowed coronary artery calcification and suppressed advanced glycation end products (AGEs) in hemodialysis patients[50]. Similarly, our previous data indicated

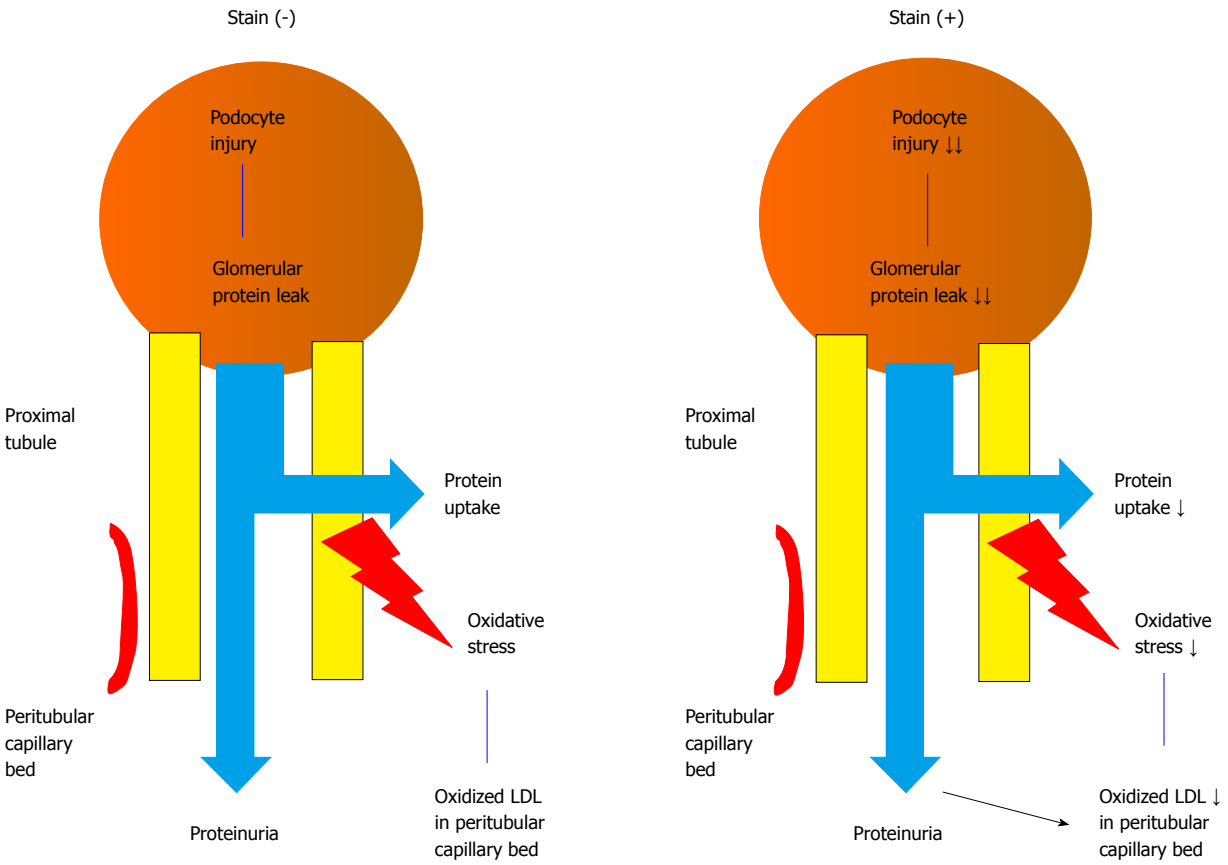

Figure 10 Multiple actions of statin on chronic kidney diseases: Statin decreases proximal tubular uptake of protein leaked from glomeruli, reducing oxidative stress. Statin also improve podocyte injury, reducing glomerular protein leak[27]. LDL: Low-density lipoprotein.

that switching from calcium carbonate to sevelamer hydrochloride reduced LDL-C levels and attenuated progressive increases in PWV in hemodialysis patients[51]. Another vicious cycle appears to link oxidative stress and AGEs[52]. AGEs induce ROS in vascular cells, leading to ongoing AGE formation and atherogenesis. Therefore, a blockade of ROS or AGE formation might interrupt this vicious cycle. In contrast to the inverse association between 25-hydroxyvitamin D and hypertension risk, 1,25-dihydroxyvitamin D was positively associated with risk of hypertension[53]. Thus, careful supplementation of vitamin D is mandatory for CKD patients. These observations suggest that an appropriate treatment for hyperphosphatemia would cut this vicious cycle and arrest further increases in arterial stiffness (especially aortic root stiffness) and central hemodynamic deteriorations in patients with stage 5 CKD.

CONCLUSION

It is not possible to determine the exact central blood pressure from brachial blood pressure. However, central blood pressure is a stronger predictor of CV and renal diseases, compared with brachial blood pressure, and should therefore be used to guide antihypertensive therapy. In CKD patients, the arteries, including the aorta, become stiff even at early stages of disease[54]. As the proportions of elderly citizens are increasing within populations, the prevalence of CKD might also increase. For these patients, central blood pressure measurements and subsequent therapeutic interventions could improve their renal and CV prognoses. However, even Western medicine remains far from meeting this goal. We hope that this review will enlighten all individuals with an interest in medical care, including medical staff members, nephrologists and cardiologists, to the details of this issue.

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ORIGINAL ARTICLE

Retrospective Study

Incidence and prevalence of hepatitis B and hepatitis C viruses in hemodialysis patients in Lebanon

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Author contributions: Abou Rached A was the major investigator who designed the study and contributed to data analysis and paper writing; El Khoury L, El Imad T and Jreijiry J collected the data and contributed to data analysis and paper writing; Geara AS performed the statistical analysis; Ammar W supervised the study and contributed to results analysis.

Institutional review board statement: This study is retrospective, involving anonymous clinical data without affecting the patient's rights and welfare. These data were obtained directly from the ministry of public health registries in collaboration with its general director. No IRB approval was required.

Informed consent statement: We performed a retrospective study using anonymous patients data collected through the ministry of public health which routinely compiles all HBsAG and HCV serology results from the affiliated HD centers across Lebanon on a monthly basis. Since then, no informed consent was required.

Conflict-of-interest statement: No funding has been received for the conduct of this study. There were no financial or personal interests to be disclosed.

Data sharing statement: All data will be made available alongside the article when published. No additional data are available.

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Abstract

AIM: To determine the incidence and the prevalence of hepatitis B and C viral infections in patients on hemodialysis (HD) across Lebanon.

METHODS: We reviewed the data registry at the Lebanese Ministry of Public Health where records of monthly hepatitis B surface antigen (HBsAg) and hepatitis C virus (HCV) serology are reported from 60 affiliated HD centers across Lebanon. All patients who were on HD or who started HD between October 2010 and July 2012 were included in the study. Patients from seven HD centers were excluded due to inadequate and incomplete results reporting. During the selected

period, HBsAg and HCV serology were available for 3769 patients from 53 HD centers distributed at all Lebanese governorates. The prevalence was calculated by dividing the number of patients with positive HBsAg or HCV serology to the total number of patients. The Incidence was calculated by dividing the number of newly acquired infection to number of patients-years (p-y). Incidence rates at different governorates were compared to each other using two tailed Z test and a P value of < 0.05 was considered significant.

RESULTS: Sixty out of 3769 HD patients were found to have positive HBS Ag and 177 out of 3769 were positive for HCV Antibodies. The prevalence of hepatitis B virus (HBV) and HCV in HD patients across Lebanon was 1.6%, and 4.7%, respectively. The comparison of prevalence according to geographic distribution could not be done accurately due to the frequent shift of patients between dialysis centers at different governorates. The incidence rate was 0.27 per 100 p-y for HBV and 0.37 per 100 p-y for HCV. There was no significant difference concerning the incidence of HBV between HD centers at different governorates (all P values > 0.1), but this difference was highly significant concerning the incidence rates of HCV which occurred predominantly in the southern centers (1.47 per 100 p-y) with a P value of 0.00068 and 0.00374 when compared to Mount Lebanon (0.21 per 100 p-y) and the Northern centers (0.19 per 100 p-y), respectively.

CONCLUSION: The incidence rate of HBV and HCV is very low in the Lebanese HD centers and their prevalence is decreasing over the last two decades.

Key words: Hemodialysis; Prevalence; Hepatitis C virus; Incidence; Hepatitis B virus; Lebanon

Core tip: This is the largest and most statistically significant study addressing the prevalence and the incidence of hepatitis B virus (HBV) and hepatitis C virus (HCV) in 3769 patients on hemodialysis (HD) through 88% of all HD centers across Lebanon over a period of 22 mo. The prevalence of HBV and HCV in the studied population was 1.6%, and 4.7%, respectively. The incidence rate was 0.27 per 100 p-y for HBV, and 0.37 per 100 p-y for HCV. These values are amongst the lowest rates reported in other countries, which is most probably related to good adherence to infection control standards in the Lebanese HD centers.

Abou Rached A, El Khoury L, El Imad T, Geara AS, Jreijiry J, Ammar W. Incidence and prevalence of hepatitis B and hepatitis C viruses in hemodialysis patients in Lebanon. *World J Nephrol* 2016; 5(1): 101-107 Available from: URL: http://www.wjgnet.com/2220-6124/full/v5/i1/101.htm DOI: http://dx.doi.org/10.5527/wjn.v5.i1.101

INTRODUCTION

The susceptibility to acquire viral hepatitis during hemodialysis (HD) has several potential underlying reasons related to both the patient and the HD procedure. First, although the rate of blood products transfusions has decreased since the introduction of erythropoietin stimulating agents, HD dependent patients still subjects of recurrent transfusions. Second, HD machines and membranes are shared between different patients which increases the risk of direct blood cross contamination within one HD unit. Vaccination does not offer the same level of protection against HBV transmission in HD patients as in the general population and finally and once exposed to HBV or HCV, End stage renal disease (ESRD) patients are more prone to become chronic carriers compared to the general population[1]. Acquiring an HBV and/or HCV infection has long-term impact on morbidity and mortality of HD patients. It has been suggested that HCV seropositivity is associated with all-cause as well as cardiovascular mortality in HD patients[2]. In addition, HBV and/or HCV infection changes the clinical course and the prognosis after kidney transplantation. In Lebanon, HBV and HCV prevalence in HD patients has not been widely studied previously. The available studies were limited to few dialysis centers and date back to the late 1990's[3-5]. Reassessing the extent of the problem for both viruses establishes the infection control protocols and the general means to prevent transmission of hepatitis infection in ESRD patients on HD.

The main objective of this study is to determine the incidence and the prevalence of HBV and HCV infections in ESRD patients on HD across Lebanon. The current common practice is that ESRD patients on HD should be screened for HBV and HCV infection before the initiation of HD and monitored monthly thereafter. This serology is reported to the ministry of public health (MOPH) on a monthly basis. Our goal is that by establishing the annual incidence of HBV and HCV infection in HD patients in the different centers in Lebanon, we will be able to document how extensive is the viral hepatitis in the Lebanese HD centers. In addition, a secondary objective is to compare the incidence and the prevalence between the different Lebanese regions in order to localize a potentially high risk center.

MATERIALS AND METHODS

Each dialysis center in Lebanon is required to report monthly serology for both HBV (HBsAg) and HCV (HCV antibody) for all its HD patients.

We reviewed the MOPH registry, which compiles all Lebanese dialysis centers data, for the period extending from October 2010 to July 2012. Using this data; we conducted an assessment of the prevalence and incidence of HBV and HCV in the HD population in Lebanon.

The study population included all the patients' who

Table 1 Distribution of hemodialysis centers across Lebanon

Governorates	No. of centers present	No. of centers with available data	No. of patients studied during this period
Beirut	6	6	559
Mount Lebanon	27	24	1632
Bekaa	7	5	394
South	6	5	339
North	11	11	757
Nabatieh	3	2	88
Total	60	53	3769

Table 2 Calculated total patient months for hepatitis B

Governorates	Total number of patients	Total patient-month	Patients with positive HBsAg	Newly acquired hepatitis B
Beirut	559	7232	8	2
Mount Lebanon	1632	23955	19	3
Bekaa	394	4026	9	2
South	339	4379	5	1
North	757	13014	18	4
Nabatieh	88	1120	1	0
Total	3769	53726	60	12

HBsAg: Hepatitis B surface antigen.

underwent HD during the period extending from October 2010 to July 2012 (*i.e.*, both already established ESRD and newly diagnosed ESRD initiated on HD). We only excluded dialysis units and patients who had incomplete, or did not report data for part or for the whole studied period (7 HD centers from a total of 60 HD centers).

Incidence analysis

Since we had monthly serology and the patients were starting or stopping dialysis at different date during the studied period, we calculated the incidence using a patient-month (p-m) unit. Each HD patient was represented in the incidence analysis by the total number of months spent undergoing HD (*i.e.*, if a patient was on dialysis only for 10 mo he will be counted in the analysis as 10 p-m).

In the incidence analysis, we excluded all the patients with positive serology at the start of the studied period. For the patients who did not seroconvert during the period they were receiving HD, we counted the total months during which the patient was on HD. For patients who eventually acquired hepatitis viral infection, we only counted the total months before the acquiring infection (the period during which the patient was at risk of acquiring an infection).

Incidence (per patient-month) = (Total number of acquired infection)/(Total patient|month)

At the end of the incidence calculation, we converted the unit p-m to patient-year (p-y) by dividing the final number by 12.

Incidence (per patient-year) = [(Total number of acquired infection)/(Total patient|month)]/12

We did the statistical analysis separately for HBV and HCV.

To compare the incidence between the different governorates and since the population is independent, we used a *Z*-test to compare head to head the incidence of acquiring hepatitis viral infection between the different Governorates. We used a $P < 0.05$ with a two-tailed *Z*-test to reject the null hypothesis with a 95 percent certainty. Since our sample size was large, our analysis did fulfill the requirement of a study power more than 80%.

Prevalence analysis

We divided the total number of patient with positive serology for HBV and HCV separately by the total number of patients studied in this period of time.

Prevalence = (Total number of patients with positive serology)/(Total number of patients)

There are a total of 60 HD centers in Lebanon, fifty three (88.3%) had a complete data reporting and were included in the analysis. To evaluate the geographic differences in viral hepatitis in Lebanon, each dialysis unit was allocated to a Governorate based on its geographic location. Table 1 layout the geographic distribution of the HD units across Lebanon. The centers with missing data and not included in the analysis were 3 of 27 (11%) of the Governorate of Mount Lebanon (Haroun Hospital; Siblin Governmental Hospital and Serhal Hospital), 2 of 7 (28%) in the Bekaa (Hermel Governmental Hospital, Hraoui Governmental Hospital), 1 of 6 (16%) in South (Hammoud Hospital) and 1 of 3 (33%) in Nabatieh (Nabatieh Governmental Hospital).

RESULTS

Sixty out of 3769 HD patients studied during a 22-mo period from October 2010 to July 2012 were found to have positive HBsAg and 177 out of 3769 were positive for HCV Antibodies during anytime for this period. The Prevalence of HBV and HCV in HD patients across Lebanon was 1.6% and 4.7%, respectively.

The prevalence of HBV in HD units by governorate was distributed as follows: 1.43% for Beirut, 1.16% for Mount Lebanon, 2.28% for Bekaa, 1.47% for the South, 2.37% for the North and 1.13% for Nabatieh. The prevalence of HCV by Governorate was distributed as follows: 3.57% for Beirut, 4.47% for Mount Lebanon, 5.58% for Bekaa, 7.07% for the South, 5.01% for the North and 0% for Nabatieh. We did not analyze the difference in the prevalence between each Governorate since it is difficult to interpret such results due to the frequent shift of patient between dialysis units (*i.e.*, some patients switch HD center between seasons and relocate to Mount Lebanon or Beirut during the winter season). The geographical distribution is detailed in Tables 2 and 3.

The incidence of HBV in HD centers in Lebanon was 0.27 per 100 p-y, while for HCV it was 0.37 per 100 p-y (Table 4). No newly acquired infection for both HBV and

Table 3 Total patient-months distribution for hepatitis C

Governorates	Total number of patients	Total patient-month	Patients with positive HCV Abs	Newly acquired hepatitis C
Beirut	559	7055	20	3
Mount Lebanon	1632	22970	73	4
Bekaa	394	3797	22	2
South	339	4078	24	5
North	757	12597	38	2
Nabatieh	88	1142	0	0
Total	3769	51639	177	16

HCV: Hepatitis C virus.

Table 4 Incidence of hepatitis B and hepatitis C among hemodialysis centers in Lebanon

Governorates	Incidence of HBV (per 100 p-y)	Incidence of HCV (per 100 p-y)
Beirut	0.33	0.51
Mount Lebanon	0.15	0.21
Bekaa	0.59	0.63
South	0.27	1.47
North	0.37	0.19
Nabatieh	0	0
Across Lebanon	0.27	0.37

HCV: Hepatitis C virus; HBV: Hepatitis B virus.

HCV were observed in Nabatieh.

While comparing the incidence of HBV in HD units between different governorates in Lebanon, no statistically significant difference was found (with a *P*-value always higher than 0.05) (Table 5). In Contrast, a statistically significant difference was found between the incidence of HCV in the South (1.47 per 100 p-y) compared to Mount Lebanon (0.21 per 100 p-y) and the North (0.19 per 100 p-y) showing a higher incidence in the South with a *P*-value of 0.00068 and 0.00374 respectively (Tables 5 and 6 for a list of the different calculated *P*-values).

DISCUSSION

It is well known that HD patients are at high risk for HCV and HBV infections. In Lebanon three small studies were done concerning the prevalence of HCV in HD patients. Naman *et al*[4] reported in 1996 that the prevalence of HCV among HD in Lebanon was 27% with a high variety between the 5 centers studied (10%-39%), Abdelnour *et al*[3] reported in 1997 in various hospitals a prevalence of 16%, Abourached *et al*[5] reported in 2006, a prevalence of 13% (2.2%-38%) in 17 HD centers in Lebanon.

In this epidemiologic study covering more than 88% of the HD centers in Lebanon, the prevalence of anti-HCV antibodies in ESRD patients undergoing HD was 4.7%, showing a decrease in the prevalence of HCV among HD patients in Lebanon over the last two decades. The lowest prevalence was in Beirut (3.5%) and the highest in the South (7%). We observed a high variability among the 53 different centers studied, ranging from as low as 0% to as high as 20%.

The reduction in HCV prevalence in HD patients is a common trend across several countries and it was mainly related to the reduction in the number of transfusions in HD patients and the improvement of the laboratory screening techniques for detection of anti-HCV antibodies in blood donors. The prevalence of HCV infection in patients on HD is highly variable but clearly much higher than in the general population of the respective countries. In phase one of the Dialysis Outcomes and Practice Patterns Study (DOPPS), a prospective observational study of adult HD patients randomly selected from 308 representative dialysis facilities in France, Germany, Italy, Japan, Spain, the United Kingdom, and United States, an overall HCV prevalence of 13% was found in 8615 patients[6].

Globally the prevalence of HCV among patients undergoing HD varies from as low as 6.1% in Germany in 2002[7] to as high as 76% in Casablanca in 2005[8]. In general, North Africa and the Middle East were cited as high prevalence areas, both in the general population and in HD patients, by the WHO in 1999[9]. Previous studies from the region have reported a prevalence of anti-HCV antibodies in HD patients of 50% in Saudi Arabia in 2000[10], 19.1% in Tunisia in 1994[11], 20.2% in Turkey in 2006[12] and 34.6% in Jordan in 2007[13].

Concerning the prevalence of HBV, Abourached *et al*[5] reported on 2007 a prevalence of 2.62% (0%-6.5%) of HBsAg in 17 HD centers in Lebanon, our study showed a decrease of the prevalence to 1.6%, ranging from 1.4% in Nabatieh and Bekaa, to 2.4% in the North. This prevalence is slightly elevated than that reported in different study In Lebanon concerning the general population[14]. We observed a high variability among the 53 different centers, ranging from as low as 0% to as high as 15%.This observed prevalence is lower than that reported in the 2008 by the Saudi Centre for Organ Transplantation (SCOT) report, where HBV seropositivity was 4.6% in the Saudi HD population while among Jordanian HD patients it was 5.9%[15]. It is also lower than that reported in HD patients in other regions including Europe (4.1%), Japan (2.2%) and the United States (2.4%) during the period extending from 1996 to 2002[16]. A study sample from the DOPPS, which included 8615 adult HD patients from 308 dialysis facilities in Western Europe and the United States, reported prevalence rates for HBV infection ranging from 0% to 6.6%[17]. Studies from less developed countries estimated that the proportion of HBsAg carriers in the HD population varies from 2% to 20%[18,19].

Prospective follow up of seronegative HD patients enabled us to observe 12 newly acquired infection for HBV and 16 newly acquired infection for HCV during a twenty two months period. We observed a 0.37 per 100 p-y incidence of new HCV infections during the 22-mo observation period. The reported incidence of new HCV infections varies considerably between countries. A rate

Table 5 *P*-values for hepatitis B

HBV	Beirut	Mount Lebanon	Bekaa	South	North	Nabatieh
Beirut		$P = 0.3759$ NS	$P = 0.5562$ NS	$P = 0.8702$ NS	$P = 0.8948$ NS	$P = 0.579$
Mount Lebanon			$P = 0.1059$ NS	$P = 0.6073$ NS	$P = 0.2208$ NS	$P = 0.7086$ NS
Bekaa				$P = 0.5038$ NS	$P = 0.5709$ NS	$P = 0.4578$ NS
South					$P = 0.778$ NS	$P = 0.6159$ NS
North						$P = 0.5547$ NS
Nabatieh						

NS: Non-significant; HBV: Hepatitis B virus.

Table 6 *P*-values for hepatitis C

HCV	Beirut	Mount Lebanon	Bekaa	South	North	Nabatieh
Beirut		$P = 0.229$ NS	$P = 0.8164$ NS	$P = 0.1274$ NS	$P = 0.2598$ NS	$P = 0.4854$ NS
Mount Lebanon			$P = 0.1822$ NS	$P = 0.00068$	$P = 0.9079$ NS	$P = 0.6548$ NS
Bekaa				$P = 0.2951$ NS	$P = 0.2036$ NS	$P = 0.438$ NS
South					$P = 0.00374$	$P = 0.2346$ NS
North						$P = 0.6707$ NS
Nabatieh						

NS: Nonsignificant; HCV: Hepatitis C virus.

as low as 0.4% was observed in France from 1997 to 2000[20] but higher rates have been reported from the Mediterranean region. According to the 2008 SCOT report, the annual rate of HCV sero-conversion in Saudi HD patients was 7%-9% while in Jordan it was 2.6%[14]. The incidence rate of 0.27 per 100 p-y for HBsAg is slightly less than that reported in Europe, Japan and the United States (0.4-1.8 per 100 p-y)[17]. While comparing the incidence of HBV infection among the different Governorates, no statistically significant difference was found. In contrast, a statistically significant difference in the incidence of HCV infection was found between the South and both Mount Lebanon and the North. This interestingly high incidence of HCV infection of 1.47 per 100 p-y found in the South need to be assessed further to be able to find a potential reason for this higher incidence.

In this study we collected data from 53 centers distributed across all the six Governorates of Lebanon from the total 60 centers reporting monthly serology to the MOPH, giving us a total of 3769 patients studied over a 22-mo period. This significantly increased the statistical power and the validity of the results.

In general, the prevalence and incidence of HBV and HCV infections in HD patients are directly related to the prevalence of these infections in the general population, the quality of healthcare services in a community and the standards of infection control practices in HD units. In Lebanon, patients on maintenance HD were found to have a higher prevalence of HCV infection of 4.7% when compared to the general population since, according to available data, anti-HCV prevalence rate of 0.2% to 0.4% of the general population in Lebanon[21], but we noted a significant decrease of this prevalence in this group of patients during the last 20 years. Also the prevalence of HBV was slightly higher than the general population 2.62% *vs* 2.2%.

This higher prevalence of HBV and HCV infection in HD compared to the general population has been confirmed by several reports from different countries[22].

The incidence of HBV and HCV in HD centers in Lebanon is very low compared to others countries in the region, this can be due to the good applications of the standards of infection control practices in HD centers and the strict surveillance by the MOPH.

A notable result of this study was the significantly higher incidence of HCV found mainly in the South that may be due to variation in the degree of implementation of the universal precautions to prevent nosocomial transmission. In order to evaluate the reason for the variation between the different HD units, a more detailed evaluation of each dialysis patient should be done especially in the units with the lowest and the highest incidence for HBV or HCV. Such studies would assist in

guiding interventions aiming to reduce the occurrence of these infections and thus reduce the morbidity and mortality of the HD population in Lebanon.

Finally this study demonstrated a reduce in the prevalence of HBV and HCV infections in HD centers during the last 2 decades and a low incidence rate due to the good applications of the standards of infection controls practice.

COMMENTS

Background

End stage renal disease (ESRD) patients on hemodialysis (HD) are particularly at higher risk for acquiring hepatitis C virus (HCV) and hepatitis B virus (HBV) than the general population, due to the sharing of contaminated machines within the same center and the higher rates of blood transfusions. Such infections have a negative impact on the clinical course of ESRD causing higher rates of morbidity and mortality. Since then, it is essential to determine the prevalence and the incidence of HBV and HCV infections in HD patients in each country, then to decide accordingly about further interventions to control such infections.

Research frontiers

The prevalence of HCV and HBV in patients on HD in Lebanon was only addressed by small studies including few HD centers. This study will determine the prevalence and incidence of HCV and HBV in a significantly larger population including 3769 HD patients through 88% of all HD centers across Lebanon between October 2010 and July 2012.

Innovations and breakthroughs

Previous studies done on smaller sample sizes limited to some Lebanese HD centers reported a prevalence of 2.6% and 13%-27%, for HBV and HCV, respectively. No reports are yet available about their incidence rate. Although accurate comparison could not be done due to the different sample sizes, this study showed a reduction in HBV and HCV prevalence through HD patients in Lebanon (1.6% and 4.7%, respectively). On the other hand, their calculated prevalence and incidence rates were found to be among the lowest values reported in other countries, either in the Middle East, or in Europe and United States. Another notable result of this study was the significantly higher incidence rate of HCV in the Southern Lebanese HD centers.

Applications

This study reflects an appropriate adherence to standards of infection control in the Lebanese HD centers limiting the spread of HBV and HCV between HD patients. However, it emphasizes the need for further investigations to reestablish those standards in some centers having significantly higher incidence rate of HCV, located mainly in the South of Lebanon.

Peer-review

In this manuscript, the authors report on the prevalence and incidence of hepatitis B and hepatitis C among HD patients in Lebanon. This paper is clinically interesting.

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ORIGINAL ARTICLE

Retrospective Study

Renal and perinephric abscesses in West China Hospital: 10-year retrospective-descriptive study

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Author contributions: Liu XQ analyzed the data and wrote the paper; Wang CC collected the data; Liu YB and Liu K designed the research.

Institutional review board statement: This study was reviewed and approved by West China Hospital Sichuan University Clinical Trials and Biomedical Ethics Committee.

Informed consent statement: This analysis used anonymous clinical data that were obtained from patients who provided informed written consent before treatment.

Conflict-of-interest statement: The authors declare that there is not conflict of interest related to this study.

Data sharing statement: No additional data are available.

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Abstract

AIM: To elucidate the clinical, radiological and laboratory profiles of renal abscess (RA) and perinephric abscess (PNA), along with related treatment and outcome.

METHODS: Ninety-eight patients diagnosed with RA or PNA using the primary discharge diagnoses identified from the International Statistical Classification of Diseases and Related Health Problems Tenth Edition (ICD-10) codes (RA: N15.101, PNA: N15.102) between September 2004 and December 2014 in West China Hospital were selected. Medical records including patients' characteristics, symptoms and signs, high-risk factors, radiological features, causative microorganisms and antibiotic-resistance profiles, treatment approaches, and clinical outcomes were collected and analyzed.

RESULTS: The mean age of the patients was 46.49 years with a male to female ratio of 41:57. Lumbar pain (76.5%) and fever (53.1%) were the most common symptoms. Other symptoms and signs included chills (28.6%), anorexia and vomiting (25.5%), lethargy (10.2%), abdominal pain (11.2%), flank mass (12.2%), flank fistula (2.0%), gross hematuria (7.1%), frequency (14.3%), dysuria (9.2%), pyuria (5.1%) and weight loss (1.0%). Painful percussion of the costovertebral angle (87.8%) was the most common physical finding. The main predisposing factors were lithiasis (48.0%), diabetes mellitus (33.7%) followed by history of urological surgery (16.3%), urinary tract infections (14.3%), renal function impairment (13.3%), liver cirrhosis (2.0%), neurogenic bladder (1.0%), renal cyst (1.0%), hydronephrosis (1.0%), chronic hepatitis B (1.0%), post-discectomy (1.0%) and post-colectomy (1.0%). Ultrasound (US) and computed tomography were the most valuable diagnostic tools and US was recommended as the initial diagnostic imaging choice. *Escherichia coli* (51.4%), *Staphylococcus aureus* (10.0%) and *Klebsiella pneumoniae* (8.6%) were the main causative microorganisms. Intravenous antibiotic

therapy was necessary while intervention including surgical and nonsurgical approaches were reserved for larger abscesses, multiple abscesses, PNAs and non-responders.

CONCLUSION: Heightened alertness, prompt diagnosis, and especially proper antibiotics in conjunction with interventional approaches allow a promising clinical outcome of renal and perinephric abscesses.

Key words: Renal abscess; Causative pathogens; Perinephric abscess; Diagnosis; Antibiotic resistance; Interventional treatment; Conservative treatment

Core tip: Renal and perinephric abscesses are uncommon but potentially lethal infectious diseases and the case-fatality rates most frequently cited in previous studies are still high. However, the previous case-fatality rates need to be updated, since prompt diagnosis and appropriate therapeutic strategies have contributed to lower mortality. This article reports the characteristics of patients identified with renal or perinephric abscesses and shares the management experience and outcome in West China Hospital during the last decade.

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INTRODUCTION

Renal abscess (RA) is defined as encapsulated pus confined to the renal parenchyma and is further divided into renal cortical or corticomedullary abscess[1]. Perinephric abscess (PNA) is a collection of suppurative material located between Gerota's fascia and the renal capsule[2]. Complications of urinary tract infections (UTIs) and hematogenous seeding from primary infected sites are the common source of infection[2,3]. Additionally, rupture of renal cortical abscess or renal carbuncle can result in the formation of PNA[2].

As a result of its anatomical location and potential to spread, RA is potentially lethal and the prognosis can be poor, especially in immunosuppressed and cachectic patients[1,4]. PNA originates from hematogenous dissemination, and often has an acute presentation with pain and high spiking temperatures[2], while in most cases, PNA is notoriously silent clinically[2,3], thereby the diagnosis can be challenging[2]. It is reported that only 35%-38% of patients with PNA are correctly diagnosed at the time of admission[5,6]. The mortality rates of RA and PNA in recent series are reported to range from 1% to 14%[3,7-12], while complicated abscess may carry a higher mortality[1]. Due to the above situation, a retrospective-descriptive study was conducted with 98 relevant cases identified with RA and PNA between September 2004 and December 2014 in West China Hospital, in an attempt to recognize the disease and describe our experience with it over the past 10 years.

MATERIALS AND METHODS

The data presented in this study were obtained from medical records of patients selected using the primary discharge diagnoses identified from the International Statistical Classification of Diseases and Related Health Problems Tenth Edition (ICD-10) codes (RA: N15.101, PNA: N15.102) during the last decade in our hospital. Suspected patients were diagnosed based on both clinical and radiological criteria. Abscesses $\leqslant$ 3 cm were defined as small, medium 3-5 cm, and > 5 cm large. The rule that culture findings guide selection of an antibiotic regimen was firmly followed, while before the culture results were obtained, initial empirical antibiotics were provided once a clinical diagnosis of RA and PNA was made. Antibiotics typically included piperacillin plus amikacin and metronidazole, piperacillin/tazobactam or third-generation cephalosporin plus metronidazole, or quinolones plus metronidazole. When patients had a severe condition such as sepsis or were prone to infection with extended-spectrum β-lactamase (ESBL)-producing organisms, carbapenem antibiotics (*e.g.*, imipenem/cilastatin) were also included in prescription.

Treatment modes were subdivided into two groups: Conservative treatment and interventional treatment. The latter comprised five categories: Antibiotics plus percutaneous drainage; antibiotics plus double J tube insertion; antibiotics plus nephrostomy; antibiotics plus surgical drainage; and antibiotics plus nephrectomy. Since improvement in clinical manifestations usually precedes that in radiological imaging findings, patients were mainly assessed by their clinical improvement. The clinical outcome was classified as cure, clinical improvement (mainly including remission or disappearance of initial symptoms, shrinkage of the abscess cavity upon imaging, recovery of white blood cell and neutrophil counts, and negative results for blood and urine culture), or death.

Biostatistics

The statistical methods of this study were reviewed by Liang Huang from Center of Infectious Diseases, West China Hospital of Sichuan University, Chengdu, Sichuan Province, China.

RESULTS

Patient characteristics

Among the 98 patients, there were 41 (41.8%) men and 57 (58.2%) women. The age ranged from 18 to 75 years with a mean of 46.49 ± 15.07 years. RA was observed in 68 (69.4%) patients and PNA in 30 (30.6%).

Table 1 Characteristics of patients with renal or perinephric abscesses *n* (%)

Variables	Value Total (*n* = 98)
Lumbar pain	75 (76.5)
Fever	52 (53.1)
Chills	28 (28.6)
Anorexia and vomiting	25 (25.5)
Lethargy	10 (10.2)
Abdominal pain	11 (11.2)
Flank mass	12 (12.2)
Flank fistula	2 (2.0)
Gross hematuria	7 (7.1)
Frequency	14 (14.3)
Dysuria	9 (9.2)
Pyuria	5 (5.1)
Loss of weight	1 (1.0)
Painful percussion of the CVA	86 (87.8)

CVA: Costovertebral angle.

Table 2 Blood and urine analysis

Variables	Value	
	RA	PNA
Blood analysis	64	30
WBC (109/L)	10.82 (range: 2.42-29.95)	12.40 (range: 2.68-25.45)
NEUT (%)	81.00 (range: 48.30-96.00)	79.00 (range: 49.30-94.70)
HGB (g/L)	105.75 ± 22.52	101.66 ± 20.13
BUN (mmol/L)	5.26 (range: 1.10-20.30)	5.10 (range: 2.80-23.18)
Serum creatinine (umol/L)	82.00 (range: 25.10-346.00)	86.60 (range: 49.00-560.0)
Urine analysis	64	30
No finding (%)	13 (13.3)	3 (3.1)
Hematuria (%)	47 (48.0)	23 (23.5)
Pyuria (%)	16 (16.3)	8 (8.2)
Proteinuria (%)	32 (32.7)	16 (16.3)
Leukocyturia (%)	41 (41.8)	23 (23.5)

RA: Renal abscess; PNA: Perinephric abscess; WBC: White blood cell; NEUT: Neutrophil count; HGB: Hemoglobin; BUN: Blood urea nitrogen.

Fifteen patients (15.3%) had no identifiable systemic or urological disorder that might have been involved in abscess formation, whereas for other patients, the spectrum of predisposing factors remained consistent with conventional predisposing factors: Diabetes mellitus (*n* = 33, 33.7%); lithiasis (*n* = 47, 48.0%) which included renal calculi (*n* = 32, 32.7%), ureteric calculi (*n* = 5, 5.1%), renal and ureteric calculi (*n* = 10, 10.2%); history of urological surgery (*n* = 16, 16.3%); UTIs (*n* = 14, 14.3%); renal function impairment (*n* = 13, 13.3%); liver cirrhosis (*n* = 2, 2.0%); neurogenic bladder (*n* = 1, 1.0%); and other diseases (*n* = 5, 5.1%) including one each with renal cyst, hydronephrosis, and chronic hepatitis B, post-discectomy and post-colectomy.

The most common initial symptoms were lumbar pain (*n* = 75, 76.5%) and fever (*n* = 52, 53.1%). Each grade of fever was observed: 38 ℃-39 ℃ in approximately 31.6%, 39.1 ℃-41 ℃ in approximately 20.4%, and absent or low-grade fever in 48.0%. Painful percussion of the costovertebral angle (*n* = 86, 87.8%) was the most common physical finding (Table 1). Patients with RA in this study were more inclined to experience lethargy than PNA ($P < 0.05$) and there was no statistical significance in other symptoms when compared RA with PNA ($P > 0.05$).

Laboratory data and abscess characteristics

There was no significant difference between RA and PNA in white blood cell count (W = 996.5, $P > 0.05$), neutrophil count (W = 947, $P > 0.05$), hemoglobin (W = 0.9773, $P > 0.05$), blood urea nitrogen (W = 992, $P > 0.05$) and serum creatinine (W = 1038, $P > 0.05$). Hematuria and leukocyturia were most common findings in urine test (Table 2). Of the 98 patients, 77 (78.6%) patients had a solitary abscess and 10 (10.2%) had multiple abscesses. The right side (55.1%) remained the predominant anatomical site and bilateral abscesses were found in two (2.0%) cases. The average size of RA and PNA was 6.25（range: 0.50-17.00）cm and 8.35 (range: 4.50-20.00) cm, respectively. The average size of abscess was 4.00 (range: 1.80-10.50) cm in the conservative group, and 7.65 (range: 0.50-20.00) cm in the interventional group (Table 3).

Microbiological data

The results of blood, abscess and urine culture were available for 92, 54 and 91 patients, respectively. Blood and urine cultures were positive in 13 (14.1%) and 23 (25.3%) patients, respectively, and pathogenic organisms were isolated from pus in 33 (61.1%) cases. Of all the positive cultures (*n* = 69), the most frequently isolated pathogen was *Escherichia coli* (*n* = 35, 50.7%) followed by *Staphylococcus aureus* (*S. aureus*) (*n* = 7, 10.1%), *Klebsiella pneumoniae* (*K. pneumoniae*) (*n* = 6, 8.7%), *Pseudomonas aeruginosa* (*n* = 3, 4.3%), *Candida spp.* (*n* = 7, 10.1%), *Enterobacteriaceae* (*n* = 6, 8.7%), *Entercoccus faecium* (*n* = 2, 2.9%), *Enterococcus faecalis* (*n* = 1, 1.4%) and *Aspergillus spp.* (*n* = 2, 2.9%). *E. coli* was more frequently found in patients with RA than those with PNA ($\chi^2 = 6.832$, $P < 0.01$), while there was no significant difference in the distribution of *K. pneumoniae* ($P > 0.05$), *S. aureus* ($P > 0.05$) and *Candida spp.* ($P > 0.05$) (Table 4). We detected ESBL in the isolated strains of *E. coli* in 12 (17.4%) cases and *K. pneumoniae* in one (1.4%) case. We analyzed the antibiotic resistance of *E. coli*, *S. aureus* and *K. pneumoniae* isolated from blood, abscess and urine culture (Table 5).

Imaging studies

Imaging results were available for 97 patients. Ultrasound (US) was applied in 80 cases and alone in 31 (31.6%) cases. Computed tomography (CT) was performed in 63 cases and alone in 16 (16.3%) cases. Magnetic resonance imaging (MRI) was applied in three cases and alone in one (1.0%) case. The imaging results are shown in Table 6.

Table 3 Treatment and outcome

Variables	Abscess size (cm)	Hospital stay (d)	No. of Patients (RA/PNA)	Cure (RA/PNA)	Clinical improvement (RA/PNA)	Death (RA/PNA)
Ab	4.00 (range: 1.80-10.50)	20.7	23 (19/4)	2 (2/0)	21 (17/4)	0 (0/0)
Intervention	7.65 (range: 0.50-20.00)	15.9	75 (49/26)	54 (38/16)	20 (10/10)	1 (1/0)
Ab + PCD			8	3	4	1
Ab + pigtails			2	1	1	0
Ab + nephrostomy			4	0	4	0
Ab + SD			29	21	8	0
Ab + NC			32	29	3	0
RA	6.25 (range: 0.50-17.00)		68	40	27	1
PNA	8.35 (range: 4.50-20.00)		30	16	14	0

Ab: Antibiotic; PCD: Percutaneous drainage; SD: Surgical drainage; NC: Nephrectomy.

Table 4 Causative microorganisms isolated from blood, abscess and urine culture

Variables	Culture		
	Blood (Total = 92)	Pus (Total = 54)	Urine (Total = 91)
No finding	79	21	68
Escherichia coli	8	17	10
Staphylococcus aureus	2	4	1
Klebsiella pneumoniaee	1	3	2
Pseudomonas aeruginosa	1	1	1
Other			
Enterobacteriaceae	1	3	2
Entercoccus faecium	0	0	2
Enterococcus faecalis	0	1	0
Candida	0	2	5
Aspergillus	0	2	0

Table 5 The antibiotic resistance rate of causative pathogens isolated

Variables	Causative pathogens isolated		
	Escherichia coli (n = 35)	Klebsiella pneumoniae (n = 6)	Staphylococcus aureus (n = 7)
Penicillin (%)	85.0	100.0	62.5
Levofloxacin (%)	46.2	0	50.0
Gentamicin (%)	53.3	0	50.0
Amikacin (%)	5.0	0	-
Cefotaxime (%)	61.1	50.0	50.0
Ceftriaxone (%)	63.2	50.0	-
Imipenem/cilastatin (%)	0	0	60.0
Vancomycin (%)	-	-	0
ST (%)	70.0	0	37.5

ST: Trimethoprim-sulfamethoxazole.

Treatment and outcome

The average hospitalization duration was 17 d (range 5-92 d). Of the 98 patients, 23 (23.5%) received conservative treatment and 75 (76.5%) received an interventional procedure. Fifty-seven (58.2%) patients were cured, 40 (40.8%) showed clinical improvement by the time of hospital discharge, and one (1.0%) died of multiple organ dysfunction syndrome. Interventional treatment contributed to a better clinical outcome than conservative treatment (Z = -3.897, P < 0.01). The outcome tended to be better in patients with RA than in those with PNA irrespective of the therapeutic mode (Z = -8.027, P < 0.01) (Table 3).

Table 6 Imaging results

Results	Imaging tool		
	US (Total = 80)	CT (Total = 63)	MRI (Total = 3)
Negative (%)	2 (2.0)	1 (1.0)	
Abscess (%)	23 (23.5)	37 (37.8)	1 (1.0)
Hydronephrosis (%)	26 (26.5)	8 (8.2)	
Mass (%)	13 (13.3)	11 (11.2)	2 (2.0)
Echogenic alteration (%)	11 (11.2)		
Cyst (%)	4 (4.1)	4 (4.1)	
Hematoma (%)	1 (1.0)	2 (2.0)	

US: Ultrasound; CT: Computerized tomography; MRI: Magnetic resonance imaging.

DISCUSSION

RA refers to a collection of purulent material within the kidney[13]. PNA represents an extensive infection in the perinephric space[2]. It is reported that approximately 30% of PNAs come from hematogenous dissemination[2], whereas in most cases, they result from rupture of RA[12,14]. Previous data show that > 80% of PNAs occur secondary to renal tract calculi with ascending UTIs[15].

RAs and PNAs are seen in all age groups and those aged 42.3-71.62 years were previously reported to be the dominant population[10-12,16-19]. A similar result was found in the present study. A slight predominance in women was noted, while in previous studies, the male: Female ratio was reported as 1:3-1:7[7,18,20], and a female predominance as high as 91.8% was also observed[16].

Diagnosis of RA or PNA remains challenging because the symptoms can be insidious and obscure[1,2]. Patients with RA may present with fever, chills, flank or abdominal pain, fatigue, nausea, decreased appetite, weight loss and even persistent hiccups[7,21,22]. In our study, fever was not always accompanied by chills and the high percentage of absent/low-grade fever might be explained by prior antibiotic therapy. Patients with PNA often present with anorexia, nausea and vomiting, flank

pain, flank mass, signs of sepsis, weight loss, fistula formation and urinary tract complaints[2,4,15,22]. However, patients with RA were more likely subjected to lethargy in this study.

Consistent with previous studies, lithiasis (48.0%) and diabetes mellitus (33.7%) remained the predominant risk factors in the present study[3,8,9,12,16,22]. Diabetes mellitus accounts for 33.3%-62.5% of all PNAs[2,7,23] 43.5%-47% of RAs[7,16,17] and 28%-50% of RAs and PNAs[3,7,10,11]. Anatomical malformation of the urinary tract, vesicoureteral reflux and obstructive tumors in renal polycystic disease are other previously described risk factors[1,18]. There was no significant difference between RA and PNA in white blood cell count, neutrophil count, hemoglobin, blood urea nitrogen and serum creatinine, which suggested that patients with RA and PNA shared similar inflammation reaction level and risk of renal impairment in this study.

US, CT and MRI were necessary to establish reliable preoperative diagnosis. US as the initial and classical imaging modality is utilized to measure renal size, discern focal lesions, and detect the true nature of a fluid-containing mass and obstruction of the collecting system. US is not affected by poor renal function or allergy to contrast material[24,25]. The accuracy of US in the diagnosis of RA is reported to be 70%-93%[3,23] with sensitivity and specificity of 78.2% and 88.8%, respectively[23].

CT has been documented to diagnose RA or PNA with an accuracy of 92%-96.4%[3,6], with specificity of 88%[23]. In our study, the accuracy of US and CT was 23.7% and 38.1%, respectively. However, when we combined the imaging results with clinical and laboratory data, the final diagnostic accuracy was 52.0%, and the average duration between admission and diagnosis was 2.16 d. With its convenience, accuracy, availability and low cost, US has made a major contribution to accurate and early diagnosis at our unit.

Since ascending dissemination of UTI has surpassed hematogenous dissemination and become the dominant predisposing factor[6,18], Gram-negative bacteria, especially *E. coli*, *Proteus* spp. and *K. pneumoniae* have been the most common pathogens in recent years[3,10,16,18]. In this study, *E. coli* was most frequently isolated from patients with RA than those with PNA. Polymicrobial abscesses have been increasingly frequently observed, ranging from 19.2% to 33.3% in incidence[7,9]. Two (2.0%) polymicrobial cases were found in the present study. There has been an increasing incidence of abscesses caused by fungi, especially *Candida*, particularly in immunosuppressed patients[7,12,18], and similar cases were observed in this study.

The selection of antimicrobial therapy ideally should be based on culture findings, however, there is an inevitable delay in obtaining results[2]. We recommend that empirical broad-spectrum intravenous antibiotics should be initiated for critically ill patients after admission. Once the blood or abscess fluid cultures and bacterial isolation tests are confirmed, targeted antibiotic regimens should be prescribed accordingly.

There is a consensus that small RAs may resolve with antibiotic treatment alone, and percutaneous or surgical drainage may be suitable for large RAs and PNAs. However there is a continuing argument about the proper treatment of middle-sized abscesses[3,5,7,16].

Although the option of conservative management of RAs and PNAs seems attractive and feasible and successful cases have been reported[4,16], those cases were selected and limitations in size, location and number of abscesses were obvious and in Iwamoto's case, the patient had received percutaneous drainage prior to conservative treatment[4].

In the present study, interventional approaches helped to detect the cause of disease and confirm the diagnosis, and culture of pus/aspirate/debris helped guide selection of an antibiotic regimen. The diagnostic and therapeutic value of percutaneous drainage has been confirmed since early years[26]. The application of interventional procedures contributed to a lower case-fatality rate and lower risk for intensive care unit (ICU) admission[6,9]. Patients subjected to interventional treatment achieved a better clinical outcome than those received conservative treatment and the cure rate of interventional treatment was 27 times higher than that of conservative treatment. On the other hand, patients with RA were more likely to achieve a better prognosis than those with PNA irrespective of the therapeutic regimens.

The mean duration of hospitalization in the interventional treatment group was 15.9 d compared with 20.7 d in the conservative treatment group. In 2011-2014, the bed turnover time in the urological department of our hospital was 9.687, 9.623, 8.92 and 8.62 d, respectively. In consideration of the pressure relating to bed turnover time, interventional treatment could be a better alternative mode to meet the social needs.

Several limitations should be noted in the present study. First, the number of patients selected was not large enough, and exclusive reliance on the claims data might have resulted in potential bias. A larger population-based retrospective-descriptive study is needed to extrapolate better and confirm our results. Second, the imaging tools were not manipulated by the same technician, thus there is inevitable error in the imaging results obtained. Finally, the number of causative microorganisms isolated from pus/blood/urine was small. In fact, the isolation rates of ESBLs of *E. coli* and *K. pneumoniae* (excluding ICU) in our hospital in 2013 were 59.8% and 29.7%, respectively. The antibiotic resistance rate of *E. coli* to penicillin, cefotaxime, gentamicin, amikacin, trimethoprim-sulfamethoxazole and imipenem/cilastatin was 88.9%, 60.7%, 44.8%, 3.0%, 56.9% and 0.7%, respectively. The antibiotic resistance rate of *K. pneumoniae* was 78.8%, 26.2%, 16.6%, 4.9%, 25.2% and 1.5%, respectively. The antibiotic resistance rate of *S. aureus* to penicillin, gentamicin, sulfamethoxazole and vancomycin was 94.1%, 29.3%, 20.5% and 0%, respectively.

Since RA and PNA can be lethal[3,6], to reduce the fatality rate when clinical suspicion is around, we recommend that physicians use US for primary evaluation and proceed to CT for confirmation. For small abscesses, intravenous antibiotics alone seem efficient. Interventional regimens are the first-line treatment for larger RAs, multiple abscesses, PNAs, and non-responders. The mode of therapy for medium-sized abscesses should depend on an individual basis, with due consideration of the clinical scenario and risk factors. However, interventional treatment is more capable of offering a promising clinical outcome and better bed turnover time and social benefits.

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COMMENTS

Background

Renal and perinephric abscesses are severe complications of urinary tract infections. Since their symptoms are insidious, the diagnosis can be challenging. Awareness combined with efficient imaging and laboratory results contribute to timely diagnosis, and appropriate treatments can lead to a good outcome and low mortality.

Research frontiers

Conservative treatment is currently reported to be practical for perinephric abscess (PNA) or larger renal abscess (RA) in certain cases, but in most cases, interventional treatment remains the classical therapy.

Innovations and breakthroughs

This study collected 98 patients diagnosed with RA or PNA in West China Hospital during the past decade. The clinical and laboratory profiles of these patients were described and analyzed. The study revealed the local epidemiological features of RA and PNA, and advocated interventional treatment for PNA and large or medium-sized RA.

Applications

This study sorted the clinical and laboratory data of RA and PNA, aiming to help strengthen the awareness of physicians and share the management experience.

Terminology

Extended-spectrum β-lactamase is an enzyme that can hydrolyze β-lactam antibiotics, including penicillins and cephalosporins. Bacteria that can produce extended-spectrum β-lactamase are resistant to β-lactam antibiotics.

Peer-review

The manuscript presents interesting data regarding the renal and perirenal abscesses for a 10-year period.

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META-ANALYSIS

Sex bias in response to hepatitis B vaccination in end-stage renal disease patients: Meta-analysis

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Abstract

AIM: To systematically review the literature for studies investigating the potential effect of gender of dialysis patients on the immunogenicity of hepatitis B virus vaccines.

METHODS: Literature searches were conducted by the MEDLINE and Google Scholar. The key words used included "hepatitis B (HB)", "vaccine", "dialysis", "hemodialysis", "sex", "male" and "female". Data of seroresponse to HB vaccine in clinical trials regarding sex of the recipients have been achieved and analyzed. Finally data from 19 clinical trials have been pooled and analyzed.

RESULTS: Analysis of response to HB vaccination in our dialysis population showed males significantly respond less to hepatitis B vaccination (P = 0.002, Z = 3.08) with no significant heterogeneity detected [P = 0.766; heterogeneity χ^2 = 14.30 (df = 19); I^2 = 0%]. A reanalysis of the pooled data was conducted regarding the dialysis mode to evaluate potential differential impact of sex on HB vaccine response. Hemodialysis was the only subgroup that showed a significant difference regarding dialysis mode in response to HB vaccination regarding sex (P = 0.042, Z = 2.03).

CONCLUSION: This Meta-analysis showed significant effect for the sex of chronic kidney disease and dialysis patients on the immunogenicity of HB vaccine. This sex discrimination was most prominent among hemodialysis patients.

Key words: Hepatitis B virus vaccination; Hepatitis B virus; Immunogenicity; Dialysis patients; Gender; Sex

Core tip: This study showed that gender of the dialysis patients is a significant factor affecting serresponse to hepatitis B vaccination (HBV) in the immunocompromised population of hemodialysis population. This gender bias was most significantly prominent when patients were under hemodialysis (*vs* other renal replacement therapies including peritoneal dialysis). The relevance of such a finding is to enable the practitioners to be alerted on the effects of HBV vaccinations in dialysis patients and give them clues to individualize vaccination protocols for patients with specific epidemiological characters.

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INTRODUCTION

Hepatitis B virus (HBV) infection is one of the most widespread chronic viral infections in the world with two billion people infected worldwide, and a matter of substantial amounts of financial and health burden throughout the world[1]. The significance of HBV infection in dialysis setting is even higher, because of the high rate of infection due to contaminations, transfusions and injections, and also the high rate of associated survival disadvantage[2]. To tackle this problem in this population, hygienic precautions have been developed whose effectiveness has been very well established[3]. Nevertheless, despite all the precautions, there are still a relatively large proportion of dialysis patients who develop the infection[4]. For the same reason, hepatitis B vaccination is an inevitable part of any preventive protocol that has been developed and proposed by health societies for the dialysis setting[5].

As mentioned, vaccination against HBV infection, though very effective, has not thoroughly eradicated the infection in the dialysis patients[6]. It has been shown that seroconversion due to HBV vaccination in dialysis patients is not perfect; and systematic reviews have shown that there are a number of factors adversely affecting response rate to HBV vaccination in dialysis patients. Erythropoietin use, diabetes mellitus, dialysis mode, vaccine administration mode, adjuvant use, vaccine type (recombinant *vs* plasma-derived), and the effect of age and nutritional status of dialysis patients on the immunogenicity of HBV vaccine are among them. Considering these factors, in a previous paper we proposed individualization of HBV vaccination in dialysis patients based on the epidemiology of the associated factors in their patient population. In the current paper, we systematically review the existing literature for studies investigating the potential effect of sex of dialysis patients on the immunogenicity of HBV vaccines in their patient population.

MATERIALS AND METHODS

Search strategy and data acquisition

The literature has been searches through the National Library of Medicine's (MEDLINE) database, and Google Scholar; the latter database has been particularly used to find relevant citations of the trials of interest; as well, specific journals have been searched to identify all the associated evidence. The key words used included "hepatitis B", "vaccine", "dialysis", "hemodialysis", "haemodialysis", "peritoneal dialysis", "gender", "sex", "male" and "female". The search has also been repeated using the reference lists of the associated systematic reviews and meta-analyses. There was no restriction in regard to the time of publication for our searches; and all the studies fulfilling the inclusion criteria were included into the analysis, irrespective of their publication year.

Inclusion and exclusion criteria

We used a number of inclusion criteria for the found studies in this systematic review: (1) they had to be available as full text (wherever the full text was not available, we contacted the corresponding author with a kind requests for the full text papers); and (2) their data is presented in a form that could be used construct a database for meta analysis were considered eligible for inclusion. There was no restriction regarding the type of vaccines employed in the trials and they were included into the meta-analysis if their vaccine was either plasma-derived or recombinant DNA preparations. The administered dosages or follow up times or vaccination routs were also not subjects to any preferable inclusion or exclusion. Studies were excluded if: (1) they reported not data on response to HBV vaccination separately for either gender in term of epidemiology of seroconversion for either gender groups; and (2) trials were published as abstracts with no enough methodology description.

End point

The association of the gender of dialysis patients has been associated with seroresponse to HB vaccine in the included trials. In cases both seroprotection and seroconversion had been reported by the included trials, seroconversion has been used as the end-point.

Source of support

This meta-analysis was not supported by any pharmaceutical company. The source of support in this study is a grant from Baqiyatallah University of Medical Sciences, Tehran, Iran.

Literature review

After excluding studies not fulfilling inclusion criteria, 19 clinical trials[7-25] have been remained whose demo-

Table 1 Basic demographic data of the included clinical trials

Study ID	First author	Ref.	Year of publish	Country of origin	Participant number	Dialysis mode
1	Abdul N Khan	[7]	1996	United States	97	HD and CAPD
2	Kai Ming Chow	[8]	2010	China	87	CAPD
3	Ismail Hamdi Kara	[9]	2004	Turkey	34	HD
4	Baris Afsar	[10]	2009	Turkey	188	HD
5 (ID) 6 (IM)	Andre F Charest	[11]	2000	Canada	97	HD
7	Yao-Lung Liu	[12]	2005	Taiwan	69	HD and CAPD
8	Nancy M Waite	[13]	1995	Canada	77	HD
9	Salwa Ibrahim	[14]	2006	Egypt	29	HD
10	Shih-Yi Lin	[15]	2012	Taiwan	156	HD and CAPD
11	Dede sit	[16]	2007	Turkey	64	HD
12	Gerald DaRoza	[17]	2003	Canada	165	CKD
13	Jamshid Roozbeh	[18]	2005	Iran	62	HD
14	Khalid Al Saran	[19]	2014	Saudi Arabia	144	HD
15	Kevin S Eardley	[20]	2002	United Kingdom	105	HD
16	Sabahattin Ocak	[21]	2008	Turkey	49	HD
17	EO Morais	[22]	2007	Brazil	70	CKD
18	Sh Taheri	[23]	2005	Iran	125	CKD (32), HD (93)
19	Carol Dacko	[24]	1996	United States	32	CAPD
20	Gerald M Fraser	[25]	1994	United States	59	HD and CAPD

CAPD: Continuous ambulatory peritoneal dialysis; HD: Hemodialysis; ID: Intra-dermal; IM: Intramuscular.

Table 2 Demography of the participants in the studies included in the meta-analysis

Author	Ref.	Age (mean ± SD)	Gender male (%)	Duration of dialysis (mo)
Abdul N Khan	[7]	47 ± 14 (CAPD) 51 ± 18 (HD)	26(55%; CAPD) 26 (52%; HD)	18 ± 23 (CAPD) 56 ± 73 (HD)
Kai Ming Chow	[8]	60 ± 11	51/87 (59)	5.8 (median)
Ismail Hamdi Kara	[9]	44 ± 15	19 (56)	27 ± 15
Baris Afsar	[10]	NA (for total)	66 (35)	NA (for total)
Andre F Charest	[11]	52 ± 2 (ID) 46 ± 2 (IM)	73 (75)	3.4 ± 1.0 (ID) 4.8 ± 2.0 (IM)
Yao-Lung Liu	[12]	52 ± 16 (CAPD) 61 ± 11 (HD)	28 (41)	43 ± 33 (CAPD) 60 ± 49 (HD)
Nancy M Waite	[13]	NA (for total)	49 (64)	NA (for total)
Salwa Ibrahim	[14]	46 ± 11	19 (66)	80 ± 59
Shih-Yi Lin	[15]	NA(for total)	64/156(41)	NA
Dede sit	[16]	NA (for total)	31 (48)	NA (for total)
Gerald DaRoza	[17]	60 ± 15	106 (46)	NA
Jamshid Roozbeh	[18]	NA(for total)	37/62 (60)	NA
Khalid Al Saran	[19]	51 ± 15	78/66 (54)	40
Kevin S Eardley	[20]	61 ± 13	58/47 (55)	18
Sabahattin Ocak	[21]	54 ± 13	56/30 (65)	30 ± 18
EO Morais	[22]	54.5 (median)	40 (57)	26
Sh Taheri	[23]	50 ± 17	77 (62)	NA
Carol Dacko	[24]	NA (for total)	19 (59)	NA (for total)
Gerald M Fraser	[25]	NA (for total)	117 (58)	NA

SD: Standard deviation; CAPD: Continuous ambulatory peritoneal dialysis; HD: Hemodialysis; NA: Not available; ID: Intra-dermal; IM: Intramuscular.

graphic data is summarized in Table 1. Demographic data of the 1709 dialysis patients reported in the 19 published papers included in this meta-analysis is presented in Table 2. Details of the vaccination approaches employed in the studies is summarized in Table 3.

Statistical analysis

The Meta analysis has been performed using a random-effects approach. Test of heterogeneity between the studies has been assessed using the I^2 statistics, which describes the proportion of total variation across studies that is the result of heterogeneity rather than chance. Statistical heterogeneity was present, defined as $P \leqslant 0.05$ or $I^2 > 50\%$. All statistical analyses was conducted using "metan" user-written commands. The meta-analysis has been performed using software Stata v.9.0 (Stata corp, TX, United States).

RESULTS

Patient characteristics

Demographic and clinical characteristics of the included

Table 3 Vaccination information details in the included clinical trials

Author	Ref.	Vaccination mode	Vaccine type	Vaccine dose	Schedule (mo)
Abdul N Khan	[7]	IM	Recombinant (Engerix-B)	40 mcg	0, 1, (2), 6
Kai Ming Chow	[8]	IM	Recombinant (Engerix-B)	40 mcg and 80 mcg	0, 1, 6
Ismail Hamdi Kara	[9]	IM	Recombinant (Engerix-B)	40 mcg	0, 1, 2, 6
Baris Afsar	[10]	IM	Recombinant	-	0, 1, 2, 6
Andre F Charest	[11]	ID and IM	Recombinant (Engerix-B)	40 mcg (IM); 5 mcg (ID)	0, 1, 2, 6
Yao-Lung Liu	[12]	IM	Recombinant (Engerix-B)	40 mcg	0, 1, 2, 6
Nancy M Waite	[13]	IM	Recombinant (Engerix-B)	40 mcg	0,1,2,6
Salwa Ibrahim	[14]	IM	Recombinant (Engerix-B)	40 mcg	0, 1, 2, 6
Shih-Yi Lin	[15]	IM	Recombinant (Engerix-B)	40 mcg	0, 1, 2, 6
Dede sit	[16]	IM	Recombinant (Hepavax)	40 mcg	0, 1, 2, 6
Gerald DaRoza	[17]	IM	Recombinant and plasma derived	20, 40 and 80 mcg	0, 1, 6
Jamshid Roozbeh	[18]	IM and ID	Recombinant (Herberbiovac-HB)	40 mcg (IM); 20 mcg (ID)	0, 1, 4
Khalid Al Saran	[19]	IM	Recombinant (Engerix-B)	40 mcg	0, 1, 2, 6
Kevin S Eardley	[20]	IM	Recombinant (Aventis MSD)	40 mcg	0, 1, 2, 12
Sabahattin Ocak	[21]	IM	Recombinant (Euvax-B)	40 mcg	0, 1, 2, 6
EO Morais	[22]	ID	Recombinant (Greencross)	2 × 5 mcg	16 injection within 8 wk
Sh Taheri	[23]	IM	Recombinant (Havana)	40 mcg	0, 1, 6
Carol Dacko	[24]	IM	Recombinant (Engerix)	40 mcg	0, 1, 2, 6
Gerald M Fraser	[25]	NA	Recombinant (Engerix-B)	20 mcg	0, 1, 2, 6

ID: Intra-dermal; IM: Intramuscular.

trials have been summarized in Table 1. All of the included clinical trials were published in English and the date of publication ranged from 1994 to 2014. Eight out of the nineteen studies (42%) were from the Middle East [Turkey (4), Iran (2), Saudi Arabia and Egypt each one study] and the remaining were from Canada (3 studies), United States (3 studies), China and Taiwan (3 studies), and United Kingdom and Brazil (1 study, each). In 10 (52.6%) studies, all patients were under hemodialysis while in two (10.5%) only patients under continuous ambulatory peritoneal dialysis (CAPD) was investigated, in 2 (10.5%) patients were chronic kidney disease (CKD) not on renal replacement therapy, in one study patients were either on maintenance hemodialysis or CKD not on dialysis, and in the remaining 4 (21%) studies, both of the dialysis modes were used.

Mean age of the participants in the included cohorts ranged from 44 to 61 years, mean duration of dialysis also ranged from 3.4 to over 80 mo and gender distribution ranged from 35% to 75% in favor of males (Table 2). In two of the studies intradermal mode of vaccination has been used besides the intramuscular mode, and in one study only intradermal mode of vaccine administration had been used. In only one study, some of the patients received plasma-derived vaccines, while in all others, the vaccine was recombinant productions. In 13 trials with intramuscular administration of the vaccine, 40 mcg had been prescribed in all patients, in one study either 40 or 80 mcg was used, and in one another 20, 40 or 80 mcg were used for vaccination. Intradermal administration of vaccine was used in doses ranging from 5 mcg to 20 mcg in different trials. One study had not declared mode of vaccine administration. Schedule of vaccination in four of the studies was 3 times (with different time intervals) and in the others but one, were a 4-times schedule (0, 1, 2, 6). In the remaining one trial, patients either received a 3 or 4 times vaccine administration schedule.

Summary of outcome

Analysis of response to HB vaccination in our dialysis population showed a significant relation to their gender with females significantly responding a better response to vaccination (P = 0.002, Z = 3.08; Figure 1). As well no significant heterogeneity has been detected in the analysis of the included studies [P = 0.766; heterogeneity χ^2 = 14.30 (df = 19); I^2 = 0%].

Reanalysis regarding dialysis mode

Then, a reanalysis of the pooled data was conducted regarding the dialysis mode to evaluate potential differential impact of gender on HB vaccine response. Hemodialysis was the only subgroup that showed a significant difference regarding dialysis mode in response to HB vaccination regarding gender and in other subgroups, gender was not discriminatory factor in vaccine response (Figure 2; HD group: P = 0.042, Z = 2.03; CAPD group: P = 0.136, Z = 1.49; HD/CAPD group: P = 0.618, Z = 0.5; CKD group: P = 0.302, Z = 1.03; CKD/HD group: P = 0.448, Z = 0.76).

Reanalysis regarding vaccination schedule

Again, the data had been reanalyzed regarding potential effect of vaccination schedule between the patient groups on the differential vaccine response regarding gender of the patients. Despite a relatively lower p value achieved for schedule "4 times vaccination", none of the subgroups showed any significant difference (Figure 3; "4 times vaccination" group: P = 0.055, Z = 1.92; "3 times vaccination" group: P = 0.088, Z = 1.71; "others" group: P = 0.393, Z = 0.86).

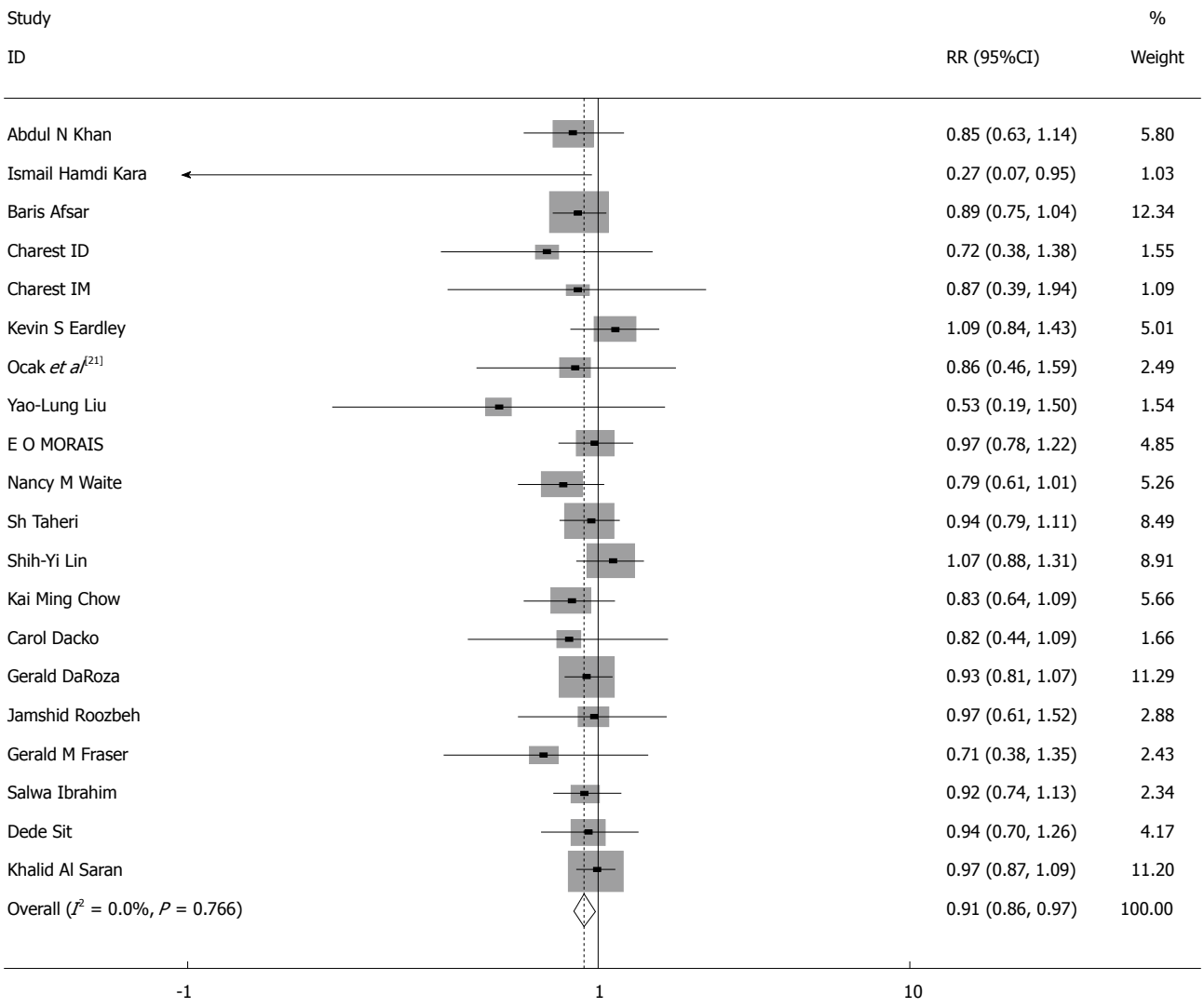

Figure 1 Forest plot: Meta-analysis of the association between gender of the end-stage renal disease patients and seroresponse to hepatitis B vaccination.

Reanalysis regarding vaccine type

The data then had been reanalyzed after removing the only trial in which a plasma-derived vaccine had been used, in order to censor potential effects of vaccine type on the study results. Nonetheless, the findings didn't change significantly ("Recombinant vaccine" group: P = 0.014, Z = 2.47; "Recombinant or plasma-derived vaccines" group: P = 0.288, Z = 1.06).

DISCUSSION

In the dialysis setting, HBV vaccination has been confirmed as an essential part of immunization, and guidelines proposed by several experts as well as health organizations almost universally recommended this procedure for this patient population[5,26,27]. These recommendations are despite the fact that patients with advanced kidney diseases have compromised immune system function, and cannot well respond to any immunization attempt made through vaccination.

The impaired immunogenicity in renal disease patients has been explained by different mechanisms, most notably impaired cellular immunity system in this population[28-30]. However, clinical trials have also proposed several other factors having predictive values in this era; but due to the controversial evidence provided by different reports, systematic reviews and meta-analyses have been conducted to pool data of all the published trials to provide a thorough conclusion from the cumulative data. Most of the published systematic reviews on this subject have been performed by Fabrizi *et al*[31] investigating potential effects of a large number of factors on HBV vaccination in dialysis patients. For example they found no significant effects for using erythropoetin (Epo)[31] and some other adjuvants[32] on the immunogeneity of HB vaccination in kidney disease patients; while several other factors significantly associated with seroconversion have also been reported by the same authors that included use of levamisole[33], granulocyte macrophage-colony stimulating factor[32] and thymopentin use[34]. Seroresponse of patients on maintenance hemodialysis *vs* peritoneal dialysis

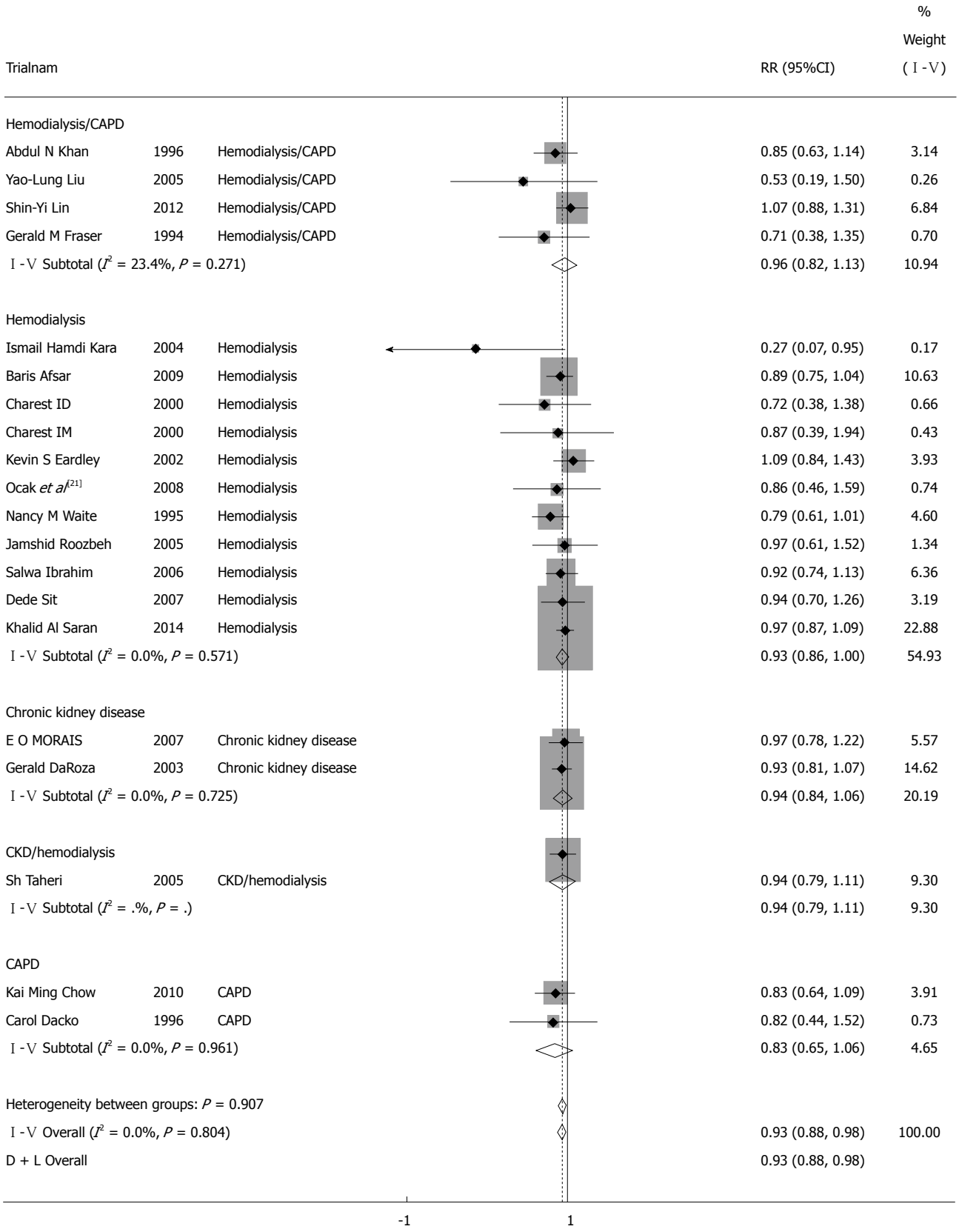

Figure 2 Forest plot: Meta-analysis of the association between gender of the end-stage renal disease patients and seroresponse to hepatitis B vaccination in patients with different therapy modality.

showed no significant difference[35]; whereas intradermal (*vs* intramuscular) administration of HB vaccine had been associated with a significantly higher vaccine response[36]. Diabetes mellitus[37] and older age[38] were

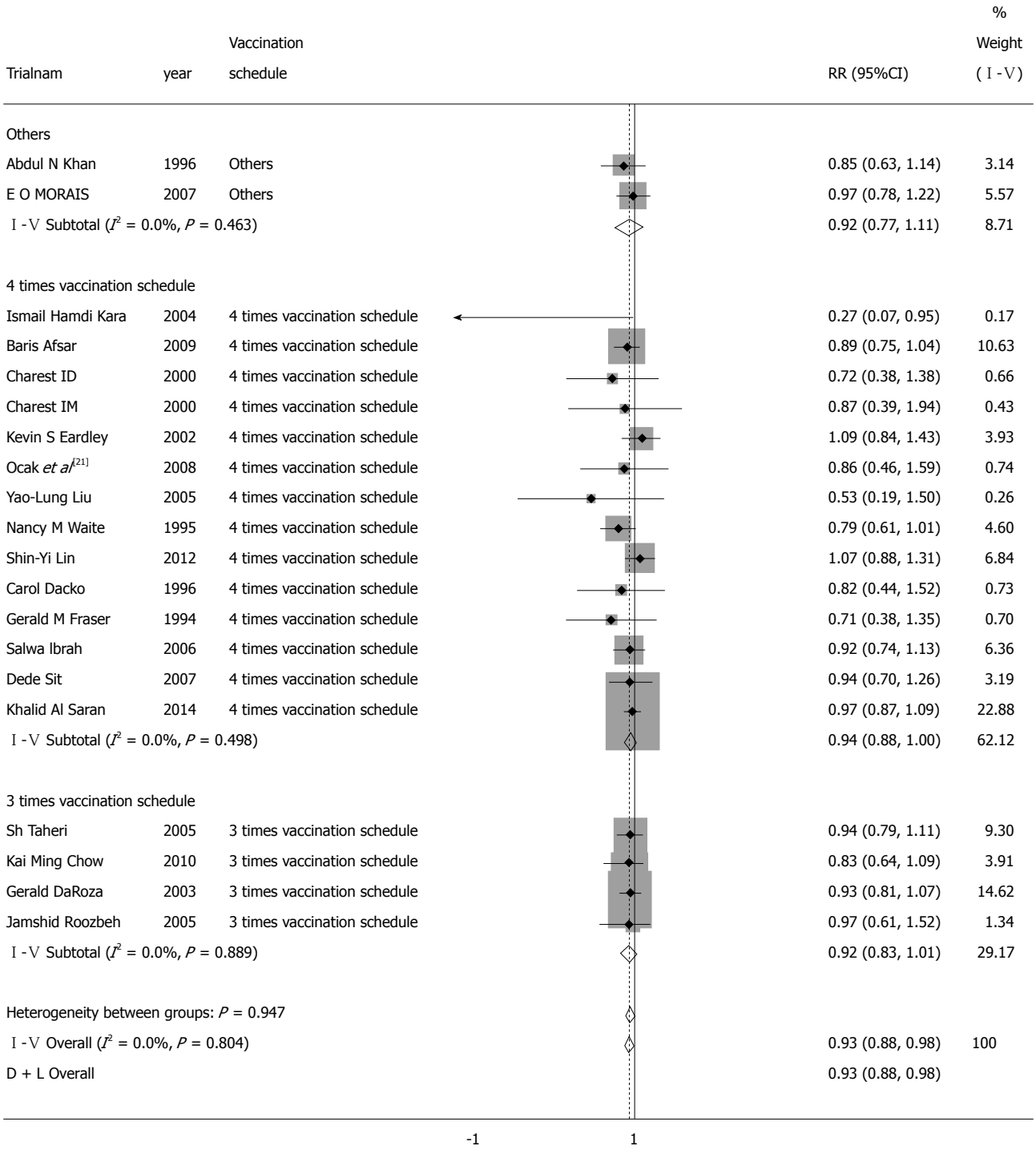

Figure 3 Forest plot: Meta-analysis of the association between gender of the end-stage renal disease patients and seroresponse to hepatitis B vaccination in patients with different vaccination schedules.

also significantly associated with poorer response to HB vaccination.

Very limited data coming from the previous clinical trials proposes that gender is a major interfering factor in the context of HB vaccine immunogenicity[9]. On the other hand, most of the existing clinical trials represent no significant role for gender on response to HB vaccination, either in kidney disease patients[7,10] or other end-stage organ disease patients[39]. However, the patient population in each of the clinical trials was limited, and in case there is a delicate difference in seroresponse to HB vaccine between the two genders, it can be easily lost. In fact, looking to most of the included clinical trials, males had relatively but not statistically significantly less percentages of response rate to HB vaccination[10,13]. This urged us to conduct this meta-analysis to pool the existing data to represent a universal outlook to the issue.

This meta-analysis showed that in the kidney disease setting, males significantly represent lower

seroconversion due to HB vaccination than females. This finding is of clinical relevance. In a previous study, it had been proposed that immunization against HB in dialysis patients should be individualized based on factors that significantly affect seroresponse in these patients[6]. So, according to the data derived from the current meta-analysis, male patients should be more rigorously surveyed after HB vaccination in dialysis setting. Moreover, future studies are recommended to find more potent immunization programs especially in this vulnerable population.

For having a more precise view on the subject, the data has been reanalyzed after stratifying the included trials based on their patients' dialysis mode, and found that the observed sex bias in the seroconversion due to HB vaccine was only significant in hemodialysis patients, and no significant difference has been observed for patients on peritoneal dialysis or CKD patients not on dialysis. Although on one hand this finding may urge us to pay more attention in men under maintenance hemodialysis therapy, we should have in mind that lack of detecting any sex discrimination in other study groups may be simply due to the comparatively limited sample size in the latter groups.

Once again, the data has been stratified based on their vaccination schedule, mainly in patients receiving 3 or 4 doses of vaccination. Although in none of the two schedules any significant difference in the seroresponse to HB vaccination has been detected regarding patients' sex, those on 4 times vaccination schedule represented a *P* value of 0.055 for sex; which might be of some value for some investigators.

Although this study is of some limitations, we believe that the findings of this study add significantly to the literature, and helps specialists to monitor their kidney disease patients more effectively and protect them against HBV infection attainment. This systematic review represents the strongest evidence on the significance of sex on the seroresponse to HB vaccination in kidney disease patients with males having more impaired immune response to the vaccination. Moreover, this sex bias was significantly more prominent among hemodialysis (*vs* other therapeutic procedures) patients, and in those on 4 times vaccination schedule (*vs* 3 times), although the latter failed to reach the significance level. It should also be mentioned that the age range of the included patients in the current meta-analysis (44-61 years) is much younger than the general age of the dialysis population, which might put some limitations in the globalization of our study results. In conclusion, this Meta analysis showed significant effect for the sex of CKD and dialysis patients on the immunogenicity of HB vaccine, with a better response for females. This sex discrimination was most prominent among hemodialysis patients. This finding suggests us to specify a sex-dependent vaccine dosage administration for patients with kidney disease. Future studies directing to find strategies with more efficacy, as well as surveys directing to find other interfering factors in this regard are recommended.

COMMENTS

Background

Dialysis patients are substantially at higher risk of developing hepatitis B virus (HBV) infection, so preventive measures are of extreme importance in this population. Anti-HBV vaccination has been the most popular preventive strategy in this population for a long time; nonetheless, its feasibility in this population has been under serious doubt. Several factors have been documented as players of significant roles in the seroresponse to HBV vaccination.

Research frontiers

During the past decades, several surveys have been performed to unveil the potential associations between dialysis patients demographic data and their seroresponse to HBV vaccination. Moreover, several systematic reviews as well as meta analyses were published to investigate these associations using pooled data of the randomized trials. To the authors' knowledge, this is the first meta-analysis that have ever investigated an citation between dialysis patents gender and their seroconversion rate after HBV vaccination.

Innovations and breakthroughs

Based on the current meta-analysis, gender is a significant factor determining response to HBV vaccination in kidney disease patients, with females significantly better responding to the vaccination. This may led future scientists to develop some individualized vaccination protocols that improve the response rate of the males to the vaccination.

Applications

Sex is a significant factor predicting seroresponse to HBV vaccination. Cumulation of data of different factors playing roles in this context can help authors to develop specific vaccination protocols for specific groups that maximizes immunization rate in this population.

Terminology

Hemodialysis is a type of renal replacement therapy which purifies the blood from unwanted materials in a way similar to kidney function. Peritoneal dialysis is a type of renal replacement therapy that uses peritoneal space for purification of the blood contents using dialysates getting injected into it. Chronic kidney disease patients are those who have significant renal function disturbance without a need to renal replacement therapy.

Peer-review

The paper is well-written and the results have potential clinical applications.

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