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FRONTIER

Cathelicidin a potential therapeutic peptide for gastrointestinal inflammation and cancer

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Abstract

Cathelicidins, are host defense peptides synthesized and stored in circulating leukocytes and numerous types of epithelial tissues in particular the gastrointestinal (GI) tract and skin. They have been known for their antimicrobial activities against a variety of microbes. Recently it was discovered that they have other significant biological functions and produce appealing pharmacological actions against inflammation and cancer in the GI tract through defined mechanisms. Experimental evidence shows that these actions could be tissue and disease specific and concentration dependent. This article reviews some of the physiological functions of cathelicidins and also their therapeutic potential in the treatment of inflammation and cancer and also the delivery system for this peptide as targeted therapy for various disorders in the GI tract both in animals and humans.

Key words: Cathelicidin; Gastrointestinal tract; Cancer; Inflammation; Ulcer repair

Biography

Professor Chi Hin Cho received his under- and post-graduate trainings in Taiwan, Canada, United States and Hong Kong and obtained his PhD in Pharmacology from the University of Hong Kong (HKU) in 1978. He had postdoctoral training in Canada and started his first faculty position in the Yang Ming Medical College and Veterans General Hospital, Taiwan in 1981. He returned to Hong Kong and joined HKU in the Department of Pharmacology, Faculty of Medicine from 1984 and became Chair Professor of Pharmacology in 2000. He joined the Chinese University of Hong Kong (CUHK) in 2007 as a chairman of the Department of Pharmacology. Currently, he is the Professor of Pharmacology and Associate Director of the School of Biomedical Sciences, Faculty of Medicine in CUHK. Professionally he was the President (2006-2010) and is now the Chair of Presidential Council (2012-2014) of the Gastrointestinal Pharmacology Section of the International Union of Basic and Clinical Pharmacology and visiting and honorary professors of Peking University, Fudan University, Zhejiang University, Beijing Capital University of Medical Science, the Fourth Military Medical University, Virginia Tech, University of Maryland and University of California. His current research interests focus on drug development for inflammation and cancers in the gastrointestinal (GI) tract. His recent work in the discovery of novel peptides including small peptides and cathelicidin as shown in this review have promising potential for drug targeting against inflammatory and cancerous diseases in the stomach and colon. These findings received prominent recognition, had significant impact on biomedical and clinical sciences and attracted international pharmaceutical industry's interest in the development of drugs and agents for the treatment and diagnosis of GI disorders. Professor Cho trained more than 50 PhD and master students and 11 postdoctoral fellows so far in his academic career. He is also the editorial board member and editor in more than 30 journals in the fields of Gastroenterology and Pharmacology. He published more than 355 peer-reviewed articles and 48 reviews in scientific journals and is the editor of six books in GI ulcer and cancer. He also holds 2 patents related to therapeutic agents for GI disorders in CUHK.

Core tip: Cathelicidin is one of the most important host defense peptides known today. It carries multiple and yet unique biological functions against pathogens which contribute to the induction and also progression of infection, inflammation and cancer, the three major types of diseases in mankind. Deficiency of such peptide would cause multiple dysfunctions in the body. In this review we highlight the physiological role and therapeutic potential of cathelicidin in inflammation and cancer and also mucosal repair in the gut. All these information would shed new lights on the development of cathelicidin as therapeutic agent for different disorders in the gastrointestinal tract.

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INTRODUCTION

Cathelicidins are innate immunity peptides. They are antimicrobial peptides (AMPs) that are produced by organisms as part of the defensive mechanism against various pathogenic microbes in humans and animals[1,2]. This class of pleiotropic peptides provides the first-line defense against infection by eliminating pathogens. Each AMP is encoded by a distinct gene. They show a great diversity in structures but have some common features, including: (1) relatively small molecular sizes (usually less than 50 amino acid residues); (2) cationic nature; (3) amphipathic helix structure; and (4) a substantial portion of hydrophobic amino acids[2,3]. Human cathelicidin (LL-37) consists of a long amphipathic helix spanning residues 2-31 with the C-terminal residues 32-37 unstructured. Another feature is that the structure is curved with a train of hydrophobic side chains. Such a cationic structure is perfect to associate with anionic micelles[4]. Indeed the cationic cathelicidin reacts electrostatically with anionic membrane components in particular cancer cells and microbes to disrupt cell membranes and induce cell death, while normal cells are neutral[5,6]. This specific property would enable cathelicidins directly and selectively attack membranes of microbes and cancer cells but spare the normal cells[7]. This uniqueness would make these peptides naturally exist and relatively non-toxic to normal mammalian system and have significant clinical implications as therapeutic agents for various diseases in particular those bacterial-related inflammation and cancer in the gastrointestinal (GI) tract[1,4,8].

CATHELICIDIN IN THE GI TRACT

Cathelicidins, a family of host defense peptides naturally expressed by cells of the GI tract. LL-37 is the mature form of human cathelicidin. It is produced constitutively by differentiated surface and upper crypt epithelial cells in the colon and by the Brunner glands in the duodenum[9]. In normal stomach, the expression of the peptide is restricted to differentiated surface of various types of cells including epithelial cells, chief cells and parietal cells and is also present in the gastric secretion. They are upregulated during infection, inflammation and wound healing both in animals and humans[9-12]. These biological responses to external challenges could have significant implications as a host defense in protection against different disorders in the GI tract.

One good example is in the course of *Helicobacter pylori* (*H. pylori*) infection in which the expression of LL-37 is induced along the gastric glands. Induction of LL-37 may help to fight against bacterial infection at the early stage. However, the expression of LL-37 is dysregulated during *H. pylori*-associated gastric carcinogenesis. During the progression from atrophic gastritis to adenocarcinoma, the expression of LL-37 is reduced[12]. All these findings indicate that cathelicidin could play a significant role in preventing bacteria related inflammation and perhaps also carcinogenesis in the GI tract. It is envisaged that deficiency of this host defense peptide could facilitate the formation of inflammation and cancer.

CATHELICIDIN AND GI REPAIR

Wound repair is a crucial adaptation to tissue damage. Based on the above information it comes to no surprise that soluble peptides like cathelicidins could evolve to orchestrate wound healing in response to mucosal damage in the GI tract. Along this line, LL-37 and mouse cathelicidin (mCRAMP) are strongly expressed in skin epithelium during wound healing in humans and mice[13]. In addition, the expression of LL-37 is low or absent in chronic ulcers, and antibodies to this peptide inhibit post-wounding re-epithelialization[14].

Induction of angiogenesis by cathelicidin further highlights its potential role in wound repair[15]. In this context, it has been proposed that the healing-promoting effect of the peptide may be mediated through modification of growth factor and receptor interactions[16,17]. However the exact mechanisms by which cathelicidins promote wound healing have not yet been fully clarified. A recent study conducted by Yang *et al*[11] in 2006 showed that rat cathelicidin can promote gastric ulcer healing in rats through induction of cell proliferation and angiogenesis. The same peptide stimulates cultured gastric epithelial cells through a transforming growth factor α-dependent transactivation of epidermal growth factor and its related pathway to induce proliferation of gastric cells[11].

CATHELICIDIN AND INFLAMMATION

Experimental evidence shows that cathelicidin can modulate inflammation by altering cytokine response and chemoattraction of inflammatory cells in diseased tissues[1,18,19]. A recent study demonstrates that bacterial DNA

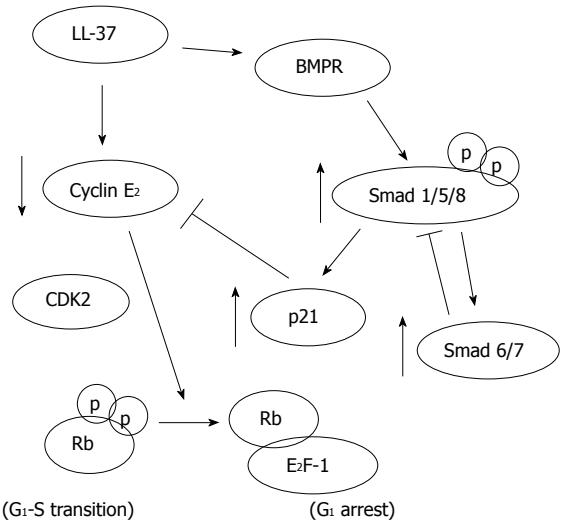

Figure 1 The possible signal pathway activated by cathelicidin (LL-37) to inhibit cell proliferation in gastric cancer cells. BMPR: Bone morphogenetic protein.

upregulates cathelicidin expression *via* a Toll-like receptor and mitogen-activated protein kinases/Erk pathway in colonic murine mucosa[20]. Clinical study also showed that cathelicidin expression was altered in inflammatory bowel diseases (IBD) patients. It was increased in both inflamed and non-inflamed mucosa in ulcerative colitis (UC) patients but not in Crohn's disease patients. The distribution of cathelicidin was also changed. Cathelicidin mainly expressed in the upper crypt of colons in healthy people in contrast to the basal part in IBD patients[21]. In another study, deficiency of cathelicidin in mCRAMP-knockout mice present more severe symptoms and mucosal disruption than the wild-type mice in response to dextran sulfate sodium challenge to induce UC. The inflammatory cytokines and the number of apoptotic cells are increased together with mucus secretion and gene expression are impaired. All these abnormalities are reversed by intrarectal administration of mCRAMP or mCRAMP-encoding plasmid[22]. On the other hand the increase of endogenous cathelicidin by agents such as butyrate and vitamin D has been suggested to modulate inflammatory responses either induced by chemical or bacteria in colonic cells[21,23-26]. Indeed butyrate treatment has been demonstrated to improve rectal histopathology in humans and eradicate Shigella *in vitro*[23,24] and vitamin D can prevent mucosal injury in chemical-induced acute colitis in mice[25].

The peptide also significantly reduces the increased number of fecal microflora in UC animals[27]. Indeed exogenous cathelicidin modulates *Clostridium difficile* (*C. difficile*) colitis. In addition, *C. difficile*-induced colitic mice treated with cathelicidin inhibits toxin A-associated intestinal inflammation[28]. In view of the current UC therapies mainly focus on relieving the inflammatory responses or reducing the pathogenic microbes, cathelicidin would have both actions, and it further promotes the mucosal defensive mechanism through mucus secretion *via* a MAP kinase pathway[29]. All these actions would provide us a better therapeutic option in the treatment of inflammation in the colon. In this context, Cho and his group develop a new form of transporting system for this peptide by combining a probiotic *Lactococcus lactis* with cathelicidin gene into a single preparation. This preparation given orally instead of intrarectal administration[22] produces similar protection against UC in mice[30]. In a similar approach, we have applied the same mCRAMP-secreting strain of *Lactococcus lactis* to reduce *H. pylori* density in the stomach as well as the associated inflammatory cell infiltration and cytokine production[31]. These findings show the feasibility of using the transformed food-graded probiotic to deliver cathelicidin to the diseased organs and exert targeted therapy. This new biological preparation would have significant clinical applications in the future as potential therapeutic agent to alleviate inflammation induced by *H. pylori* infection in the stomach and bacteria overgrowth in the colon.

CATHELICIDIN AND CANCER

Although studies have demonstrated that LL-37 could promote tumorigenesis in some cancers including lung and breast cancers as well as epithelial ovarian cancer[32-34]. Other reports have shown that LL-37 may induce cell death in many tissues. In human airway epithelial cells, LL-37 has been shown to result in apoptotic TUNEL positive cells in a caspases-dependent manner[35]. Analogue of LL-37 could induce the caspase-independent apoptosis in an oral squamous cell line SAS-H1 but not normal cells[36]. The anti-tumorigenic effect of LL-37 is dependent on its ability to induce DNA break and mitochondrial damage in Jurkat T leukemia and A549 cells which are independent of caspase activation[37]. It is likely that low tissue expression of LL-37 could promote tumor formation. Indeed downregulation of LL-37 in cancer tissues has also been reported in the GI tract. In normal gastric mucosa, LL-37 is expressed in surface epithelial cells and chief cells as well as parietal cells in the fundic glands. Immunochemical staining of LL-37 has revealed that the expression of LL-37 is down-regulated in gastric hyperplastic polyps, tubular adenomas, and adenocarcinomas[12]. After *H. pylori* infection, LL-37 is markedly up-regulated in the epithelium and gastric secretions. Such upregulation could not be detected in patients with *H. pylori*-independent gastric inflammation. Moreover, a higher level of LL-37 expression has been demonstrated in wild-type *H. pylori* infection of cultured gastric epithelial cells and this higher production of LL-37 requires an intact type Ⅳ secretion system[4,12]. Therefore, it is indicated that expression of LL-37 may be in a tissue- and disease-specific manner.

Our recent study shows that LL-37 may function as a putative tumor-suppressing gene in gastric carcinogenesis. We found that exogenous LL-37 inhibits proliferation and induces G_0/G_1-phase cell cycle arrest through a defined signal pathway in gastric cancer cells (Figure 1). Furthermore depletion of endogenous LL-37 stimulates gastric cancer cell DNA synthesis suggesting that the

Table 1 Possible functional effects and mechanisms of action of cathelicidins in different gastrointestinal disorders

Type of GI disorders	Functional effects	Mechanisms	Ref.
Ulcer	Increases of cell proliferation, re-epithelialization and angiogenesis	Activation of growth factors and their receptors	[11,13-17]
Inflammation	Decrease of pathogenic microbes, inflammatory cytokines and apoptosis; increase of mucus secretion	Activation of MAP kinase, formyl peptide receptor and mucin genes; electrostatic interaction on microbial membrane	[1,5,8,23]
Cancer	Induction of apoptosis and cell cycle arrest	Release of AIF/EndoG; activation of BMPR and Smads	[29,30]

GI: Gastrointestinal; MAP: Mitogen-activated protein; AIF: Apoptosis-inducing factor; BMPR: Bone morphogenetic protein.

antiproliferative effect of LL-37 occurs at physiological concentrations. The direct anti-cancer action has also been confirmed in a gastric xenograft cancer model in nude mice[38]. In the lower GI tract, it has been shown that LL-37 is strongly expressed in the human normal colon mucosa but downregulated in colon cancer tissues. In both settings it is correlated with the number of apoptotic cells in colonic mucosa. To this end, the pro-apoptotic activity of LL-37 is confirmed in colon cancer cells in which the peptide activates a GPCR-p53-Bax/Bak/Bcl-2 signaling cascade that triggers off the AIF/EndoG-mediated apoptosis in colon cancer cells[39]. All these findings suggest that cathelicidin could be a tumor suppressor gene in the stomach and colon. Supplementation of which would have a great potential as a therapeutic agent for gastric and colon cancers.

PERSPECTIVES AND CONCLUSION

The host defense peptide cathelicidin is highly expressed in the GI mucosa. This peptide and its recombinant protein in a deliverable preparation represent an appealing option for the treatment of inflammation and cancer and also promotion of mucosal repair in the GI tract (Table 1). This is especially true for those diseases associated with bacteria including gastritis and UC. Depletion of cathelicidin by unknown epigenetic mechanisms in the gastric and colonic tissues could be one of the causative factors in the promotion of inflammation and carcinogenesis in both organs. Supplementation with this host defense peptide orally through an effective delivery system seems to be a promising approach to treat different disorders in the GI tract.

REFERENCES

1 **Wu WK**, Wong CC, Li ZJ, Zhang L, Ren SX, Cho CH. Cathelicidins in inflammation and tissue repair: Potential therapeutic applications for gastrointestinal disorders. *Acta Pharmacol Sin* 2010; **31**: 1118-1122 [PMID: 20676121 DOI: 10.1038/aps.2010.117]

2 **Zasloff M**. Antimicrobial peptides of multicellular organisms. *Nature* 2002; **415**: 389-395 [PMID: 11807545 DOI: 10.1038/415389a]

3 **Hancock RE**, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat Biotechnol* 2006; **24**: 1551-1557 [PMID: 17160061 DOI: 10.1038/nbt1267]

4 **Wu WK**, Wang G, Coffelt SB, Betancourt AM, Lee CW, Fan D, Wu K, Yu J, Sung JJ, Cho CH. Emerging roles of the host defense peptide LL-37 in human cancer and its potential therapeutic applications. *Int J Cancer* 2010; **127**: 1741-1747 [PMID: 20521250 DOI: 10.1002/ijc.25489]

5 **Schweizer F**. Cationic amphiphilic peptides with cancer-selective toxicity. *Eur J Pharmacol* 2009; **625**: 190-194 [PMID: 19835863 DOI: 10.1016/j.ejphar.2009.08.043]

6 **Guaní-Guerra E**, Santos-Mendoza T, Lugo-Reyes SO, Terán LM. Antimicrobial peptides: general overview and clinical implications in human health and disease. *Clin Immunol* 2010; **135**: 1-11 [PMID: 20116332 DOI: 10.1016/j.clim.2009.12.004]

7 **Boman HG**. Antibacterial peptides: basic facts and emerging concepts. *J Intern Med* 2003; **254**: 197-215 [PMID: 12930229 DOI: 10.1046/j.1365-2796.2003.01228.x]

8 **Ahluwalia A**, Tarnawski AS. Cathelicidin gene therapy: a new therapeutic option in ulcerative colitis and beyond? *Gene Ther* 2013; **20**: 119-120 [PMID: 22456327 DOI: 10.1038/gt.2012.23]

9 **Hase K**, Eckmann L, Leopard JD, Varki N, Kagnoff MF. Cell differentiation is a key determinant of cathelicidin LL-37/human cationic antimicrobial protein 18 expression by human colon epithelium. *Infect Immun* 2002; **70**: 953-963 [PMID: 11796631 DOI: 10.1128/IAI.70.2.953-963.2002]

10 **Wong CC**, Zhang L, Ren SX, Shen J, Chan RL, Cho CH. Antibacterial peptides and gastrointestinal diseases. *Curr Pharm Des* 2011; **17**: 1583-1586 [PMID: 21548872 DOI: 10.2174/138161211796197025]

11 **Yang YH**, Wu WK, Tai EK, Wong HP, Lam EK, So WH, Shin VY, Cho CH. The cationic host defense peptide rCRAMP promotes gastric ulcer healing in rats. *J Pharmacol Exp Ther* 2006; **318**: 547-554 [PMID: 16670350 DOI: 10.1124/jpet.106.102467]

12 **Hase K**, Murakami M, Iimura M, Cole SP, Horibe Y, Ohtake T, Obonyo M, Gallo RL, Eckmann L, Kagnoff MF. Expression of LL-37 by human gastric epithelial cells as a potential host defense mechanism against Helicobacter pylori. *Gastroenterology* 2003; **125**: 1613-1625 [PMID: 14724813 DOI: 10.1053/j.gastro.2003.08.028]

13 **Dorschner RA**, Pestonjamasp VK, Tamakuwala S, Ohtake T, Rudisill J, Nizet V, Agerberth B, Gudmundsson GH, Gallo RL. Cutaneous injury induces the release of cathelicidin anti-microbial peptides active against group A Streptococcus. *J Invest Dermatol* 2001; **117**: 91-97 [PMID: 11442754 DOI: 10.1046/j.1523-1747.2001.01340.x]

14 **Heilborn JD**, Nilsson MF, Kratz G, Weber G, Sørensen O, Borregaard N, Ståhle-Bäckdahl M. The cathelicidin anti-microbial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium. *J Invest Dermatol* 2003; **120**: 379-389 [PMID: 12603850 DOI: 10.1046/j.1523-1747.2003.12069.x]

15 **Koczulla R**, von Degenfeld G, Kupatt C, Krötz F, Zahler S, Gloe T, Issbrücker K, Unterberger P, Zaiou M, Lebherz C, Karl A, Raake P, Pfosser A, Boekstegers P, Welsch U, Hiemstra PS, Vogelmeier C, Gallo RL, Clauss M, Bals R. An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. *J Clin Invest* 2003; **111**: 1665-1672 [PMID: 12782669 DOI: 10.1172/JCI200317545]

16 **Gallo RL**, Ono M, Povsic T, Page C, Eriksson E, Klagsbrun M, Bernfield M. Syndecans, cell surface heparan sulfate proteo-

glycans, are induced by a proline-rich antimicrobial peptide from wounds. *Proc Natl Acad Sci USA* 1994; **91**: 11035-11039 [PMID: 7972004 DOI: 10.1073/pnas.91.23.11035]
17 **Li J**, Post M, Volk R, Gao Y, Li M, Metais C, Sato K, Tsai J, Aird W, Rosenberg RD, Hampton TG, Sellke F, Carmeliet P, Simons M. PR39, a peptide regulator of angiogenesis. *Nat Med* 2000; **6**: 49-55 [PMID: 10613823]
18 **Zanetti M**. Cathelicidins, multifunctional peptides of the innate immunity. *J Leukoc Biol* 2004; **75**: 39-48 [PMID: 12960280 DOI: 10.1189/jlb.0403147]
19 **Metz-Boutigue MH**, Shooshtarizadeh P, Prevost G, Haikel Y, Chich JF. Antimicrobial peptides present in mammalian skin and gut are multifunctional defence molecules. *Curr Pharm Des* 2010; **16**: 1024-1039 [PMID: 20030614 DOI: 10.2174/138161210790963823]
20 **Koon HW**, Shih DQ, Chen J, Bakirtzi K, Hing TC, Law I, Ho S, Ichikawa R, Zhao D, Xu H, Gallo R, Dempsey P, Cheng G, Targan SR, Pothoulakis C. Cathelicidin signaling via the Toll-like receptor protects against colitis in mice. *Gastroenterology* 2011; **141**: 1852-1863.e1-3 [PMID: 21762664 DOI: 10.1053/j.gastro.2011.06.079]
21 **Schauber J**, Rieger D, Weiler F, Wehkamp J, Eck M, Fellermann K, Scheppach W, Gallo RL, Stange EF. Heterogeneous expression of human cathelicidin hCAP18/LL-37 in inflammatory bowel diseases. *Eur J Gastroenterol Hepatol* 2006; **18**: 615-621 [PMID: 16702850]
22 **Tai EK**, Wu WK, Wang XJ, Wong HP, Yu L, Li ZJ, Lee CW, Wong CC, Yu J, Sung JJ, Gallo RL, Cho CH. Intrarectal administration of mCRAMP-encoding plasmid reverses exacerbated colitis in Cnlp(-/-) mice. *Gene Ther* 2013; **20**: 187-193 [PMID: 22378344 DOI: 10.1038/gt.2012.22]
23 **Raqib R**, Sarker P, Bergman P, Ara G, Lindh M, Sack DA, Nasirul Islam KM, Gudmundsson GH, Andersson J, Agerberth B. Improved outcome in shigellosis associated with butyrate induction of an endogenous peptide antibiotic. *Proc Natl Acad Sci USA* 2006; **103**: 9178-9183 [PMID: 16740661 DOI: 10.1073/pnas.0602888103]
24 **Raqib R**, Sarker P, Mily A, Alam NH, Arifuzzaman AS, Rekha RS, Andersson J, Gudmundsson GH, Cravioto A, Agerberth B. Efficacy of sodium butyrate adjunct therapy in shigellosis: a randomized, double-blind, placebo-controlled clinical trial. *BMC Infect Dis* 2012; **12**: 111 [PMID: 22574737 DOI: 10.1186/1471-2334-12-111]
25 **Zhao H**, Zhang H, Wu H, Li H, Liu L, Guo J, Li C, Shih DQ, Zhang X. Protective role of 1,25(OH)2 vitamin D3 in the mucosal injury and epithelial barrier disruption in DSS-induced acute colitis in mice. *BMC Gastroenterol* 2012; **12**: 57 [PMID: 22647055 DOI: 10.1186/1471-230X-12-57]
26 **Lagishetty V**, Chun RF, Liu NQ, Lisse TS, Adams JS, Hewison M. 1-α-hydroxylase and innate immune responses to 25-hydroxyvitamin D in colonic cell lines. *J Steroid Biochem Mol Biol* 2010; **121**: 228-233 [DOI: 10.1016/j.jsbmb.2010.02.004]
27 **Tai EK**, Wu WK, Wong HP, Lam EK, Yu L, Cho CH. A new role for cathelicidin in ulcerative colitis in mice. *Exp Biol Med* (Maywood) 2007; **232**: 799-808 [PMID: 17526772]
28 **Hing TC**, Ho S, Shih DQ, Ichikawa R, Cheng M, Chen J, Chen X, Law I, Najarian R, Kelly CP, Gallo RL, Targan SR, Pothoulakis C, Koon HW. The antimicrobial peptide cathelicidin modulates Clostridium difficile-associated colitis and toxin A-mediated enteritis in mice. *Gut* 2012 Jul 3; Epub ahead of print [PMID: 22760006]
29 **Tai EK**, Wong HP, Lam EK, Wu WK, Yu L, Koo MW, Cho CH. Cathelicidin stimulates colonic mucus synthesis by up-regulating MUC1 and MUC2 expression through a mitogen-activated protein kinase pathway. *J Cell Biochem* 2008; **104**: 251-258 [PMID: 18059019 DOI: 10.1002/jcb.21615]
30 **Wong CC**, Zhang L, Li ZJ, Wu WK, Ren SX, Chen YC, Ng TB, Cho CH. Protective effects of cathelicidin-encoding Lactococcus lactis in murine ulcerative colitis. *J Gastroenterol Hepatol* 2012; **27**: 1205-1212 [PMID: 22507188 DOI: 10.1111/j.1440-1746.2012.07158.x]
31 **Zhang L**, Yu J, Wong CC, Ling TK, Li ZJ, Chan KM, Ren SX, Shen J, Chan RL, Lee CC, Li MS, Cheng AS, To KF, Gallo RL, Sung JJ, Wu WK, Cho CH. Cathelicidin protects against Helicobacter pylori colonization and the associated gastritis in mice. *Gene Ther* 2012 Dec 20; Epub ahead of print [PMID: 23254369 DOI: 10.1038/gt.2012.92]
32 **Coffelt SB**, Waterman RS, Florez L, Höner zu Bentrup K, Zwezdaryk KJ, Tomchuck SL, LaMarca HL, Danka ES, Morris CA, Scandurro AB. Ovarian cancers overexpress the antimicrobial protein hCAP-18 and its derivative LL-37 increases ovarian cancer cell proliferation and invasion. *Int J Cancer* 2008; **122**: 1030-1039 [PMID: 17960624 DOI: 10.1002/ijc.23186]
33 **Heilborn JD**, Nilsson MF, Jimenez CI, Sandstedt B, Borregaard N, Tham E, Sørensen OE, Weber G, Ståhle M. Antimicrobial protein hCAP18/LL-37 is highly expressed in breast cancer and is a putative growth factor for epithelial cells. *Int J Cancer* 2005; **114**: 713-719 [PMID: 15609314 DOI: 10.1002/ijc.20795]
34 **von Haussen J**, Koczulla R, Shaykhiev R, Herr C, Pinkenburg O, Reimer D, Wiewrodt R, Biesterfeld S, Aigner A, Czubayko F, Bals R. The host defence peptide LL-37/hCAP-18 is a growth factor for lung cancer cells. *Lung Cancer* 2008; **59**: 12-23 [PMID: 17764778 DOI: 10.1016/j.lungcan.2007.07.014]
35 **Lau YE**, Bowdish DM, Cosseau C, Hancock RE, Davidson DJ. Apoptosis of airway epithelial cells: human serum sensitive induction by the cathelicidin LL-37. *Am J Respir Cell Mol Biol* 2006; **34**: 399-409 [PMID: 16340000 DOI: 10.1165/rcmb.2005-0170OC]
36 **Okumura K**, Itoh A, Isogai E, Hirose K, Hosokawa Y, Abiko Y, Shibata T, Hirata M, Isogai H. C-terminal domain of human CAP18 antimicrobial peptide induces apoptosis in oral squamous cell carcinoma SAS-H1 cells. *Cancer Lett* 2004; **212**: 185-194 [PMID: 15279899 DOI: 10.1016/j.canlet.2004.04.006]
37 **Aarbiou J**, Tjabringa GS, Verhoosel RM, Ninaber DK, White SR, Peltenburg LT, Rabe KF, Hiemstra PS. Mechanisms of cell death induced by the neutrophil antimicrobial peptides alpha-defensins and LL-37. *Inflamm Res* 2006; **55**: 119-127 [PMID: 16673155 DOI: 10.1007/s00011-005-0062-9]
38 **Wu WK**, Sung JJ, To KF, Yu L, Li HT, Li ZJ, Chu KM, Yu J, Cho CH. The host defense peptide LL-37 activates the tumor-suppressing bone morphogenetic protein signaling via inhibition of proteasome in gastric cancer cells. *J Cell Physiol* 2010; **223**: 178-186 [PMID: 20054823 DOI: 10.1002/jcp.22026]
39 **Ren SX**, Cheng AS, To KF, Tong JH, Li MS, Shen J, Wong CC, Zhang L, Chan RL, Wang XJ, Ng SS, Chiu LC, Marquez VE, Gallo RL, Chan FK, Yu J, Sung JJ, Wu WK, Cho CH. Host immune defense peptide LL-37 activates caspase-independent apoptosis and suppresses colon cancer. *Cancer Res* 2012; **72**: 6512-6523 [PMID: 23100468 DOI: 10.1158/0008-5472.CAN-12-2359]

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Relationships of CDXs and apical sodium-dependent bile acid transporter in Barrett's esophagus

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Abstract

Barrett's esophagus (BE) is characterized by intestinal metaplasia with the differentiated epithelium replaced by another type of epithelium morphologically similar to normal intestinal epithelium. The metaplasia is preceded by bile and acid reflux into the esophagus. BE is a premalignant condition associated with increased risk of esophageal cancer, especially esophageal adenocarcinoma. The Caudal-related homeodomain transcription factors Caudal-related homeodomain transcription factor CDX1 and CDX2 are expressed exclusively in the small and large intestine, playing important roles in proliferation and differentiation of intestinal epithelial cells. Ectopic expression of CDX1 and CDX2 occurs in BE. The apical sodium-dependent bile acid transporter (ASBT) is expressed primarily in terminal ileum where it is a key factor for intestinal reabsorption of bile salts. In addition to upregulation of CDX1 and CDX2, ASBT expression is up-regulated in BE. Furthermore, both CDX1/CDX2 and ASBT expressions are down-regulated in high-grade esophageal dysplasia. The alteration of the above-mentioned factors calls for attention: what is the relationship between CDXs and ASBT aberrant expression in BE? In this commentary, we discuss this issue on basis of the recent study done by Ma *et al*.

Key words: Esophagus; Intestinal metaplasia; Caudal-related homeodomain transcription factors; Apical sodium-dependent bile acid transporter; Aberrant expression

Core tip: Aberrant co-expression of Caudal-related homeodomain transcription factors (CDXs) and apical sodium-dependent bile acid transporter (ASBT) in the epithelium of Barrett's esophagus (BE) indicates association among these factors. Acid and bile reflux induce *CDXs* gene expression and can lead to formation of BE. CDX-mediated promoter activation can lead to aberrant expression of ASBT. The BE phenotype may be better than squamous epithelium to protect against refluxed acid and bile. On the other hand the BE phenotype is associated with increased risk of esophageal adenocarcinoma (EAC). Furthermore, the decreased expressions of CDXs and ASBT in high-grade esophageal dysplasia indicate that CDXs and ASBT are inhibitory factors to the progression of EAC.

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COMMENTARY ON HOT TOPICS

Recently, an interesting study by Ma *et al*[1] demonstrated that short interfering RNA-mediated knockdown of Caudal-related homeodomain transcription factors (CDXs) resulted in reduced apical sodium-dependent bile acid transporter (ASBT) mRNA expression in intestinal cells.

CDXs strongly induced activity of the ASBT promoter of esophageal and intestinal cells. Association with ASBT expression was found for CDX1, CDX2 and hepatocyte nuclear factor-1α in Barrett's esophagus (BE) biopsies. Ma *et al*[1] concluded that CDX1 and CDX2 activate the human ASBT promoter by transcription. For the first time ASBT is added to the list of genes regulated by CDXs. We strongly recommend this paper to the readers.

BE is a clinically important disease. The human esophagus is lined by a multilayered squamous epithelium which withstands the potential damage from rapidly propelling food boluses through the esophagus and also from intermittent exposure to refluxed contents from the stomach. However, the esophageal epithelium, usually at the gastroesophageal junction, can be inflamed and injured if the esophageal epithelium chronically and repeatedly is in contact with refluxed bile and acid. This can result in intestinal metaplasia where the esophageal squamous epithelium is replaced by intestinal-type epithelium, which is the key feature of BE[2]. BE is characterized not only by the morphological intestinalization but also by changes in gene expression patterns. The intestinal specific transcription factors CDX1 and CDX2 and other intestinal proteins such as villin, sucrase isomaltase, and acidic mucins/MUC2 can be detected in human BE tissue[3]. BE is an important risk factor for esophageal adenocarcinoma (EAC)[4,5]. The molecular mechanisms related to BE are not yet fully understood. Currently, it is believed that the BE cell emerges from (1) the esophageal squamous epithelium; (2) the distal esophagus submucosal gland epithelium; (3) the proximally-migrating gastric cardia epithelium; or from (4) infiltrating bone marrow stem cells[6,7]. Hence, the mechanism of BE formation is not well understood.

ASBT is a 48-kDa transmembrane protein. At the apical membrane of ileal enterocytes, ASBT is the chief mediator of active sodium-dependent intestinal bile acid absorption[8]. The roles of ASBT on bile acid reabsorption, regulation of *ASBT* gene expression and its association with some diseases have been reviewed in detail[8-11]. ASBT is mainly expressed in the terminal ileum but is also expressed in renal tubule cells, cholangiocytes, and the gallbladder[10]. It was recently shown that the expression of ASBT is elevated in esophageal epithelial cells from BE patients whereas ASBT expression was decreased in esophageal adenocarcinoma at both mRNA and protein levels[12]. CDX1 and CDX2 are expressed exclusively in the small and large intestine, playing important roles in proliferation and differentiation of intestinal epithelial cells. The role of *CDX* genes in the gut has recently been reviewed[13]. In adults, CDX1 is expressed primarily in intestinal crypts[14,15] whereas CDX2 is expressed in the paracaecal region of the intestine. Furthermore, CDX2 is expressed relatively more in the villi than in the crypts[14]. CDX1 and CDX2 are not expressed in the normal human esophagus[16]. However, CDX1 and CDX2 levels are elevated in the epithelium of BE[16,17]. CDX2 expression has been demonstrated in all biopsy specimens from BE without and with dysplasia, and from BE-associated adenocarcinomas[18,19]. Expression of CDX1 mRNA and protein was found in biopsy specimens from patients with BE but not from epithelium of normal esophagus[20]. Furthermore, similar to expression of ASBT, CDX2 expression decreased esophageal metaplasia progressed into adenocarcinoma[21,22].

The aberrant co-expression of CDXs and ASBT in the BE epithelium makes us ask what is the relationship between CDXs and ASBT, how do these factors relate to BE, BE with dysplasia and BE-associated adenocarcinomas. In order to study the relationship between CDXs and ASBT, Ma *et al*[1] conducted a test series to (1) study whether endogenous human ASBT mRNA levels are regulated by CDX1 and CDX2; (2) study the possible direct role for CDX1 and CDX2 in the regulation of the ASBT promoter; (3) identify putative CDX response elements (CDXREs) within the ASBT; (4) study whether the proximal promoter region containing the predicted CDXREs mediate the CDX-dependent activation; (5) study the potential *in vitro* interaction between CDX1 and CDX2 with their predicted binding motifs within the ASBT promoter; and to (6) confirm the interaction between CDX1 and CDX2 with the ASBT promoter also within living cells. Ma *et al*[1] found in human esophageal and intestine-derived cell lines that the human ASBT promoter is a direct target for transcriptional activation by the transcription factors CDX1 and CDX2. In other words, ASBT expression is regulated by CDXs. Therefore, their study adds a new piece of knowledge to the already known complexity of transcriptional regulation of *ASBT* gene expression. Furthermore, Ma *et al*[1] discover close association of CDX and ASBT expression levels in human BE tissue. This indicate that CDX-mediated ASBT promoter activation can lead to aberrant esophageal expression of the bile acid uptake system ASBT and consequently to an increase in epithelial bile acid uptake activity by the BE mucosa.

It is well known that BE is closely associated with gastro-esophageal reflux disease (GERD). In animal models, GERD caused increased Cdx2 expression in cells of the basal layer of esophageal squamous epithelium. The increased Cdx2 expression preceded the development of intestinal metaplasia[23,24]. In esophageal biopsy specimens from patients with BE, Vallböhmer *et al*[17] found high levels of CDX2 mRNA in specialized intestinal metaplasia. A recent study done by Kazumori *et al*[25] shows that Cdx1 is over-expressed in esophageal metaplastic tissue and that bile acids increase promoter activity in cultured esophageal epithelial cells. This in turn appears to induce production of Cdx2 sufficient to cause intestinal metaplasia. It has been proposed that bile acids in refluxed contents cause tight junctions in squamous cells to break, allowing the bile acids to leak into the basal layer, resulting in cell transdifferentiation[26].

From the above-mentioned studies it is evident that acid reflux and bile reflux contribute to increased CDX expression levels. The induction of *CDXs* genes precedes

the morphologic changes in BE. The BE phenotype may be better than squamous epithelium to protect against exposure to refluxed acid and bile. Furthermore, CDX-mediated promoter activation leads to aberrant expression of ASBT, resulting in increased epithelial bile acid uptake by the BE mucosa. However, the BE phenotype has 30-125 times increased risk of EAC compared to that of the general population[27]. Furthermore, although CDXs expression can be detected in well or moderately well differentiated EAC, expression of CDXs is decreased and may even be undetectable in poorly differentiated EAC[28,29]. Ma *et al*[1] found that ASBT like CDXs decrease its expression in high-grade esophageal dysplasia. All together this suggests that CDXs and ASBT expression have an inhibitory role for the progression of EAC. However, the exact mechanism about the effect of CDX1, CDX2 and ASBT on BE and BE-associated esophageal dysplasia is not well understood yet and need further study.

In summary, aberrant co-expression of CDXs and ASBT in BE epithelium stimulates further interest into learning more about the relationship between CDXs and ASBT and how they relate to BE. Based on reviewing the study by Ma *et al*[1] and other relevant literature, it is anticipated that *ASBT* gene expression is regulated by CDXs. Acid and bile reflux as well as inflammation induce *CDXs* gene expression preceding BE. CDX-mediated promoter activation can lead to aberrant expression of ASBT. The BE phenotype may be better than squamous epithelium to protect against refluxed acid and bile. On the other hand, BE phenotype is associated with increased risk of EAC. Furthermore, CDXs and ASBT expressions decrease in high-grade esophageal dysplasia. This indicates that CDXs and ASBT expression may be inhibitory factors for progression of EAC. Further research is needed for understanding the exact mechanism and effects of CDX1, CDX2 and ASBT on BE and BE-associated esophageal dysplasia.

REFERENCES

1 **Ma L**, Jüttner M, Kullak-Ublick GA, Eloranta JJ. Regulation of the gene encoding the intestinal bile acid transporter ASBT by the caudal-type homeobox proteins CDX1 and CDX2. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G123-G133 [PMID: 22016432 DOI: 10.1152/ajpgi.00102.2011]

2 **Stairs DB**, Kong J, Lynch JP. Cdx genes, inflammation, and the pathogenesis of intestinal metaplasia. *Prog Mol Biol Transl Sci* 2010; **96**: 231-270 [PMID: 21075347 DOI: 10.1016/B978-0-12-381280-3.00010-5]

3 **Morales CP**, Souza RF, Spechler SJ. Hallmarks of cancer progression in Barrett's oesophagus. *Lancet* 2002; **360**: 1587-1589 [PMID: 12443613]

4 **Paulson TG**, Reid BJ. Focus on Barrett's esophagus and esophageal adenocarcinoma. *Cancer Cell* 2004; **6**: 11-16 [PMID: 15261138]

5 **Reid BJ**, Li X, Galipeau PC, Vaughan TL. Barrett's oesophagus and oesophageal adenocarcinoma: time for a new synthesis. *Nat Rev Cancer* 2010; **10**: 87-101 [PMID: 20094044 DOI: 10.1038/nrc2773]

6 **Guillem PG**. How to make a Barrett esophagus: pathophysiology of columnar metaplasia of the esophagus. *Dig Dis Sci* 2005; **50**: 415-424 [PMID: 15810619]

7 **Fitzgerald RC**. Molecular basis of Barrett's oesophagus and oesophageal adenocarcinoma. *Gut* 2006; **55**: 1810-1820 [PMID: 17124160]

8 **Dawson PA**, Lan T, Rao A. Bile acid transporters. *J Lipid Res* 2009; **50**: 2340-2357 [PMID: 19498215 DOI: 10.1194/jlr.R900012-JLR200]

9 **Shneider BL**. Intestinal bile acid transport: biology, physiology, and pathophysiology. *J Pediatr Gastroenterol Nutr* 2001; **32**: 407-417 [PMID: 11396803]

10 **Alrefai WA**, Gill RK. Bile acid transporters: structure, function, regulation and pathophysiological implications. *Pharm Res* 2007; **24**: 1803-1823 [PMID: 17404808]

11 **Kosters A**, Karpen SJ. Bile acid transporters in health and disease. *Xenobiotica* 2008; **38**: 1043-1071 [PMID: 18668439 DOI: 10.1080/00498250802040584]

12 **Dvorak K**, Watts GS, Ramsey L, Holubec H, Payne CM, Bernstein C, Jenkins GJ, Sampliner RE, Prasad A, Garewal HS, Bernstein H. Expression of bile acid transporting proteins in Barrett's esophagus and esophageal adenocarcinoma. *Am J Gastroenterol* 2009; **104**: 302-309 [PMID: 19174784 DOI: 10.1038/ajg.2008.85]

13 **Beck F**, Stringer EJ. The role of Cdx genes in the gut and in axial development. *Biochem Soc Trans* 2010; **38**: 353-357 [PMID: 20298182 DOI: 10.1042/BST0380353]

14 **Silberg DG**, Swain GP, Suh ER, Traber PG. Cdx1 and cdx2 expression during intestinal development. *Gastroenterology* 2000; **119**: 961-971 [PMID: 11040183]

15 **Subramanian V**, Meyer B, Evans GS. The murine Cdx1 gene product localises to the proliferative compartment in the developing and regenerating intestinal epithelium. *Differentiation* 1998; **64**: 11-18 [PMID: 9921649]

16 **Silberg DG**, Furth EE, Taylor JK, Schuck T, Chiou T, Traber PG. CDX1 protein expression in normal, metaplastic, and neoplastic human alimentary tract epithelium. *Gastroenterology* 1997; **113**: 478-486 [PMID: 9247467]

17 **Vallböhmer D**, DeMeester SR, Peters JH, Oh DS, Kuramochi H, Shimizu D, Hagen JA, Danenberg KD, Danenberg PV, DeMeester TR, Chandrasoma PT. Cdx-2 expression in squamous and metaplastic columnar epithelia of the esophagus. *Dis Esophagus* 2006; **19**: 260-266 [PMID: 16866857]

18 **Groisman GM**, Amar M, Meir A. Expression of the intestinal marker Cdx2 in the columnar-lined esophagus with and without intestinal (Barrett's) metaplasia. *Mod Pathol* 2004; **17**: 1282-1288 [PMID: 15167938]

19 **Phillips RW**, Frierson HF, Moskaluk CA. Cdx2 as a marker of epithelial intestinal differentiation in the esophagus. *Am J Surg Pathol* 2003; **27**: 1442-1447 [PMID: 14576477]

20 **Wong NA**, Wilding J, Bartlett S, Liu Y, Warren BF, Piris J, Maynard N, Marshall R, Bodmer WF. CDX1 is an important molecular mediator of Barrett's metaplasia. *Proc Natl Acad Sci USA* 2005; **102**: 7565-7570 [PMID: 15894614]

21 **Hayes S**, Ahmed S, Clark P. Immunohistochemical assessment for Cdx2 expression in the Barrett metaplasia-dysplasia-adenocarcinoma sequence. *J Clin Pathol* 2011; **64**: 110-113 [PMID: 21106545 DOI: 10.1136/jcp.2010.075945]

22 **Villanacci V**, Rossi E, Zambelli C, Galletti A, Cestari R, Missale G, Casa DD, Bassotti G. COX-2, CDX2, and CDC2 immunohistochemical assessment for dysplasia-carcinoma progression in Barrett's esophagus. *Dig Liver Dis* 2007; **39**: 305-311 [PMID: 17307036]

23 **Pera M**, Pera M, de Bolós C, Brito MJ, Palacín A, Grande L, Cardesa A, Poulsom R. Duodenal-content reflux into the esophagus leads to expression of Cdx2 and Muc2 in areas of squamous epithelium in rats. *J Gastrointest Surg* 2007; **11**: 869-874 [PMID: 17440788]

24 **Tatsuta T**, Mukaisho K, Sugihara H, Miwa K, Tani T, Hattori T. Expression of Cdx2 in early GRCL of Barrett's esophagus induced in rats by duodenal reflux. *Dig Dis Sci* 2005; **50**: 425-431 [PMID: 15810620]

25 **Kazumori H**, Ishihara S, Kinoshita Y. Roles of caudal-related homeobox gene Cdx1 in oesophageal epithelial cells in Barrett's epithelium development. *Gut* 2009; **58**: 620-628 [PMID: 19136512 DOI: 10.1136/gut.2008.152975]
26 **Souza RF**, Krishnan K, Spechler SJ. Acid, bile, and CDX: the ABCs of making Barrett's metaplasia. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G211-G218 [PMID: 18556417 DOI: 10.1152/ajpgi.90250.2008]
27 **Shaheen NJ**, Crosby MA, Bozymski EM, Sandler RS. Is there publication bias in the reporting of cancer risk in Barrett's esophagus? *Gastroenterology* 2000; **119**: 333-338 [PMID: 10930368]
28 **Moskaluk CA**, Zhang H, Powell SM, Cerilli LA, Hampton GM, Frierson HF. Cdx2 protein expression in normal and malignant human tissues: an immunohistochemical survey using tissue microarrays. *Mod Pathol* 2003; **16**: 913-919 [PMID: 13679455]
29 **Werling RW**, Yaziji H, Bacchi CE, Gown AM. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. *Am J Surg Pathol* 2003; **27**: 303-310 [PMID: 12604886]

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REVIEW

Persistent hypertransaminasemia in asymptomatic children: A stepwise approach

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Abstract

We aimed to examine the major causes of isolated chronic hypertransaminasemia in asymptomatic children and develop a comprehensive diagnostic flow diagram. A MEDLINE search inclusive of publications throughout August 2012 was performed. We found only a small number of publications that had comprehensively investigated this topic. Consequently, it was difficult to construct a diagnostic flowchart similar to those already available for adults. In children, a "re-testing panel" prescription, including gamma-glutamyl transpeptidase and creatine kinase in addition to aminotransferases, is considered a reasonable approach for proficiently confirming the persistence of the abnormality, ruling out cholestatic hepatopathies and myopathies, and guiding the subsequent diagnostic steps. If re-evaluation of physical and historical findings suggests specific etiologies, then these should be evaluated in the initial enzyme retesting panel. A simple multistep diagnostic algorithm incorporating a large number of possible pediatric scenarios, in addition to the few common to adults, is available. Accurately classifying a child with asymptomatic persistent hypertransaminasemia may be a difficult task, but the results are critical for preventing the progression of an underlying, possibly occult, condition later in childhood or during transition. Given the high benefit/cost ratio of preventing hepatic deterioration, no effort should be spared in diagnosing and properly treating each case of persistent hypertransaminasemia in pediatric patients.

Key words: Transaminase; Aminotransferase; Hypertransaminasemia; Liver disease; Children

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INTRODUCTION

The measurement of serum transaminase levels has become part of the routine biochemical evaluation that takes place before surgery or for the investigation of pathologies not necessarily related to liver injury in many countries. An investigation of unexpected hypertransaminasemia is important for differentiating muscular and hepatic disease; to institute timely and specific treatment for progressive, but still asymptomatic, treatable liver conditions [*e.g.*, Wilson's disease, autoimmune hepatitis (AIH), and non-alcoholic fatty liver disease (NAFLD)]; to furnish genetic counseling for hereditary disorders; and/or to setup appropriate preventive measures (*e.g.*, avoidance of viral hepatitis transmission). Moreover, prevention of possible hepatic deterioration has a high benefit/cost ratio by avoiding the need for eventual liver transplantation[1].

Currently, the most frequent cause of hypertransaminasemia in both adults and children is obesity, although obesity-related liver disease is still sometimes erroneously

considered cryptogenic because of a poor perception of obesity among medical practitioners[2-4]. In adults, the causes of isolated hypertransaminasemia other than obesity-related liver disease are limited to viral hepatitis, toxic damage, autoimmune hepatobiliary diseases, celiac disease, Wilson's disease, and hereditary hemochromatosis[5,6], whose diagnostic work-up is well established[4,6-11]. The problem is more complex in children, in whom individually rare genetic/metabolic conditions collectively constitute 20%-30% of liver diseases[12,13]. Therefore, persistent hypertransaminasemia in a child should alert the physician to the possibility of an underlying hepatic or multisystem metabolic disorder and prompt a referral to a specialized center for diagnostic evaluation. However, despite extensive investigation, the etiology of some cases may have to be defined as truly unknown (cryptogenic)[12,14].

There are only a few reports examining the possible causes of isolated chronic hypertransaminasemia in asymptomatic pediatric populations[12,14-16], and often these studies are biased by flawed inclusion and exclusion criteria, and/or an inadequate diagnostic work-up. Consequently, there is no diagnostic algorithm for the differential diagnosis of unexpected chronic hypertransaminasemia in pediatric patients.

The aim of this report is to develop a comprehensive diagnostic algorithm that includes many of the frequent causes of isolated asymptomatic hypertransaminasemia in children and adolescents. We examined current adult guidelines and expert opinions, and then performed a MEDLINE search inclusive of publications throughout August 2012 using the key words: transaminase, aminotransferase, hypertransaminasemia, liver disease, and children. We also evaluated reports from specialized tertiary pediatric hepatology centers. A secondary aim was to provide a basis for future studies on the cost/benefit ratio of diagnostic assessment procedures.

TRANSAMINASES: BACKGROUND ON ORIGINS, LEVELS, AND THRESHOLD LEVELS

Aminotransferases are normally present in circulation at low levels. They are intracellular enzymes produced principally by hepatocytes, and their increase in serum is therefore indicative of liver cell injury. However, aspartate aminotransferase (AST) is also found in cardiac and skeletal muscles, the kidneys, brain, pancreas, and lungs, and in erythrocytes, in decreasing order of concentration. Additionally, alanine aminotransferase (ALT) is present in skeletal muscle and kidneys, but at low concentrations, and its increase in the circulation is more specific for liver damage than AST. Aminotransferase serum levels depend not only on the tissue of origin, but also on the enzyme half-life, which is longer for ALT than AST. Thus, in diseases such as muscular dystrophy, patients can have AST and ALT serum values that are elevated to the same degree, instead of the expected prevalent elevation of AST[17].

In clinical practice, normal parameter values are within 2 standard deviations of the mean value obtained in healthy individuals. This implies that 5% of the results of healthy subjects fall outside this range. While transaminase reference intervals for adults have recently been revised[18,19], this has not occurred for children. England *et al*[20] recently proposed ALT centiles stratified by sex and age in a healthy European population. They propose an ALT upper limit of normal of 60 IU/L in boys and 55 IU/L in girls during the first 18 mo of life. The range changes to 40 IU/L in boys and 35 IU/L in girls after the age of 18 mo. The Screening ALT for Elevation in Today's Youth (SAFETY) study conducted on a population of North American patients aged between 12 and 17 years shows that the upper limit of normal used by most laboratories for ALT is too high to detect chronic liver disease and that less than half of North American hospitals utilize gender-specific values. In that study, the ALT thresholds in use had a low sensitivity for the detection of chronic liver damage (30%-40%). Using the National Health and Nutrition Examination Survey (NHANES) ALT threshold of 25.8 IU/L for boys and 22.1 IU/L for girls, the sensitivity improved to 70%-80%, while the specificity was only reduced from approximately 90% to approximately 80%[21].

Clinical recommendation

In the pediatric population, there is no established reference range of ALT and AST. ALT thresholds currently in use have a low sensitivity for the detection of chronic liver damage. In teenagers, the biologically-determined and gender-specific ALT threshold of 25.8 U/L for boys and 22.1 U/L for girls established by NHANES increases this sensitivity, with only a modest specificity reduction.

APPROACH TO ASYMPTOMATIC HYPERTRANSAMINASEMIA

Table 1 summarizes the most frequent causes of persistently-elevated transaminase levels in asymptomatic children, schematically classified under the categories of viral, autoimmune, metabolic, and other types of hepatobiliary diseases or extrahepatic causes of hypertransaminasemia.

A detailed evaluation of medical and family history and an accurate clinical examination are crucial in determining the likely etiology of hypertransaminasemia, as they may indicate towards possible muscle or liver conditions.

Initial step: The retesting panel

A stepwise approach contemplates repeating the tests to confirm the results[5,9,10]. In adults, timing of retesting is not firmly established because it has usually been empirically guided by the degree of transaminase alterations found {mild [< 5 times upper limit of normal (× ULN)]; moderate (5-10 × ULN); marked (> 10 × ULN)}.

In pediatric patients, information about gamma-glutamyl transferase (GGT) rather than alkaline phosphatase values might help to determine whether the liver injury

Table 1 Main causes of asymptomatic hypertransaminasemia in children

Hepatic origin	Extrahepatic origin
Obesity (non-alcoholic fatty liver disease)	Duchenne/Becker muscular dystrophy (prevalence: 1:4700)
Viral infections (major and minor hepatotropic viruses)	Other myopathies (*e.g.*, caveolinopathies; prevalence: 1:14000 to 1:120000)
Autoimmune liver disease (prevalence: 1:200000)	Myocardiopathies
Celiac disease and inflammatory bowel disease	Nephropathies
Wilson's disease (prevalence: 1:30000)	Hemolytic disorders
Cystic fibrosis (prevalence: 1:2500) and Shwachman-Diamond syndrome (prevalence: 1:50000)	Macro - AST (prevalence: 30% of children with isolated aspartate aminotransferasemia)
Alpha1 antitrypsin deficiency (prevalence: 1:7000)	
Other genetic and metabolic diseases[1]	
Toxic: Drugs and alcohol	
Cryptogenic hypertransaminasemia	

[1]Refer to Table 3. AST: Aspartate aminotransferase.

is predominantly hepatocellular or cholestatic[5]. Creatine phosphokinase (CPK) should also be evaluated to rule out occult muscle disease[22].

As in adults[5,10,23], in many asymptomatic children, abnormal values can show normal values when re-tested[12,14-16,23]. The reported percentage of patients who normalize aminotransferase serum values within 6 mo from the first detection of abnormality ranges from 26% to 73.6%[12,14-16]. A fluctuating pattern (transient/self-limiting or intermittent) is frequently observed at all ages, and more than one retesting may be warranted even for a mild increase of transaminases. In areas with a high prevalence of hepatitis B (HBV) and C (HCV) virus infection and in high-risk populations, it is advisable to request viral markers at the time of repeat testing to accelerate the screening protocol[9,10]. Recently, Senadhi[24] recommended that HBV and HCV screening is warranted in all asymptomatic patients with mild transaminase elevations.

In selected patients who participate in strenuous sports, liver tests should be repeated after at least one week without exercise, especially if hypertransaminasemia was associated with high CPK or with elevation of other enzymes of muscle origin[25]. If high CPK and hypertransaminasemia are confirmed, it is mandatory to exclude muscular diseases, which are often clinically asymptomatic during the first 5-6 years of life and can be recognized only after a detailed and oriented neurologic examination[22]. In addition to the well-known muscular dystrophies, myocyte injury, necrosis induced by drugs or toxins, and increased exercise are possible causes of high CPK and hypertransaminasemia. Additionally, some mitochondrial, endocrine and metabolic myopathies, and gluten enteropathy are also causes of high CPK and hypertransaminasemia. A serum elevation of CPK ranging from 450 to 5000 U/L (normal upper limit: 150 U/L) accompanying isolated asymptomatic hypertransaminasemia can also be a marker of a caveolinopathy, a group of newly described and still poorly understood muscle diseases that affect the limb-girdle, distal muscles, and heart. A diagnosis may be particularly challenging in pediatric patients that are only mildly symptomatic[26].

First, second, and third line investigations

The evaluation of patients with confirmed hypertransaminasemia should include first (and eventually second and third) line investigations (Table 2). If the patient's history or physical examination suggests a particular disease, selected first-line investigations should already be part of the retesting panel. However, some authors suggest that first-line exams for hepatocellular causes of liver disease should be performed without the need to confirm hypertransaminasemia in patients with increased serum levels of ALT > 3-5 × ULN[27]. Historically, a hypertransaminasemia duration of approximately 6 mo has been arbitrarily used to determine chronic liver disease. However, it is unwise to wait for 6 mo before investigating a possible cause of liver damage, as some hepatopathies, such as autoimmune liver disease or Wilson's disease, can become rapidly life-threatening without appropriate treatment[28].

In the case of protracted isolated AST elevation, the possibility of macro-AST should be investigated by polyethylene glycol and/or electrophoresis testing. Macro-AST is a condition characterized by the presence in serum of a macromolecular complex formed by association with other plasma components [*e.g.*, immunoglobulin G (IgG)] or by self-polymerization. Due to their large size, macro-AST components cannot be filtered by renal glomeruli and are retained in the plasma. The condition is benign, but may cause diagnostic uncertainty and lead to useless, invasive, expensive, and time-consuming testing[29].

Clinical recommendation

Aminotransferase levels should be retested upon finding hypertransaminasemia in asymptomatic patients. At this time, the levels of CPK (for muscular disease) and GGT (for biliary involvement) should also be evaluated. An accurate clinical history and physical examination are of paramount importance for guiding an appropriate investigation and avoiding expensive and unnecessary tests.

MOST COMMON LIVER DISEASES

Non-alcoholic fatty liver disease

NAFLD is the most common cause of hypertransaminasemia in children and adolescents[12,14,16,21]. In clinical practice, NAFLD is usually suspected by the finding of hypertransaminasemia and ultrasonographic fatty liver in an obese child[30,31]. Although the ALT serum level is a useful diagnostic tool, it is not a sensitive marker of NAFLD. It is common to observe the entire histological spectrum of NAFLD in patients with normal ALT levels[22,32]. The evaluation of both AST and ALT values is important because an increased AST/ALT ratio has been reported to reflect a progressive and more serious condition [fibrotic

Table 2 Retesting panel; first, second, and third line investigations in children with asymptomatic mild hypertransaminasemia

Retesting panel[1]	First line panel		Second and third line panels
	Liver function tests	Etiology tests	
ALT	Conjugated and unconjugated bilirubin	Viral markers (HAV, HBV, HCV)	Urinary copper, molecular ATP B7 analysis
AST	Protein electrophoresis	Minor hepatotropic viruses serology	HCV RNA, HBV DNA
CPK	Serum albumin	(*e.g.*, EBV, CMV)	Genetic and metabolic enlarged screening[2] ("non-
GGT	Prothrombin time and partial	Ceruloplasmin, serum copper	alcoholic fatty liver disease bin")
	thromboplastin time	ANA, SMA, LKM, LC1, anti-SLA,	Sweat test
	Blood cell count	total IgG	Fecal elastase, steatocrit
	Hepatic ultrasonography	Serum α1 antitrypsin	Other hepatic imaging techniques (MRI, ERCP, CT, *etc.*)
	If only AST elevation is confirmed: PEG	EMA, tTgasi IgA, deamidated AGA	Liver biopsy[3]
	test and electrophoresis for macro-AST	IgA (< 2 yr), total IgA	Jejunal biopsy (after celiac disease serology)

[1]After at least one week off from exercise; [2]Genetic and metabolic enlarged screening: blood gases, lactic acid, serum ammonium concentrations, blood and urinary amino acids, urinary reducing substances, urinary organic acids, transferrin isoforms, screening test for congenital disorders of glycosylation, α1 antitrypsin phenotype; [3]Modified from the reference of 28. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; PEG: Polyethylene glycol; CPK: Creatine kinase; GGT: Gamma-glutamyl transferase; HAV: Hepatitis A virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; EBV: Epstein-Barr virus; CMV: Cytomegalovirus; ANA: Antinuclear antibodies; SMA: Smooth muscle antibodies; LKM: Anti-microsomal antibodies; LC1: Anti-liver cytosol antibodies type 1; SLA: Soluble liver antigen; IgG: Immunoglobulin G; EMA: Anti-endomysial antibodies; tTgasi: Anti-tissue transglutaminase antibodies; AGA: Anti-gliadin antibodies; IgA: Immunoglobulin A; ATP B7: ATP binding protein 7; MRI: Magnetic resonance imaging; ERCP: Endoscopic retrograde cholangiopancreatography; CT: Computed tomography.

Table 3 Fatty liver disease; possible causes in likely asymptomatic children and adolescents

General or systemic	Genetic-metabolic causes	Drugs/chemicals
Obesity	Cystic fibrosis	Ethanol
Metabolic syndrome	Shwachman syndrome	Ecstasy, cocaine, solvents
Obstructive sleep apnea	Wilson's disease	Nifedipine
Polycystic ovary syndrome	Alpha 1-antitrypsin deficiency	Diltiazem
Diabetes mellitus type 1	Fructosemia	Estrogens
Thyroid disorders	Cholesterol ester storage disease	Corticosteroids
Hypothalamic-pituitary disorders	Glycogen storage disease (type Ⅰ, Ⅵ and Ⅸ)	Methotrexate
Inflammatory bowel disease	Mitochondrial and peroxisomal defects	Prednisolone
Celiac disease	α- and β-oxidation defects	Valproate
Protein calorie malnutrition	Organic acidosis	Vitamin
Rapid weight loss	Abeta or hypobetalipoproteinemia	Zidovudine and HIV treatments
Anorexia nervosa	Porphyria cutanea tarda	Solvents
Small intestinal bacterial overgrowth	Homocystinuria	Pesticides
Hepatitis C	Familial hyperlipoproteinemias	
	Bile acids synthesis defects	
	Congenital disorders of glycosylation	
	Citrin deficiency	
	Turner syndrome	

Modified from the references of 30 and 38. HIV: Human immunodeficiency virus.

non-alcoholic steatohepatitis (NASH)][33].

Due to the worldwide obesity epidemic, obesity may be erroneously accepted as a normal condition even in the presence of abnormal liver function tests[2]. Conversely, in the presence of obesity, all other conditions associated with abnormal transaminase levels, such as celiac disease[34,35], Wilson's disease[36], autoimmune hepatitis[37], or muscular diseases[22], must be investigated and excluded. In many of these diseases, prompt treatment will avoid irreversible disease progression[38] (Table 3).

Abdominal ultrasound and liver function tests should be performed as a first step in the diagnosis of possible NAFLD in children[39]. Ultrasonography, however, detects fat levels above 30% only. Waist circumference is a valid surrogate marker of central obesity, which is closely correlated with liver involvement[40]. When pediatric NAFLD is suspected, other liver diseases should be excluded based on an age-driven algorithm[39]. In obese children and adolescents with disordered breathing during sleep, sleep apnea syndrome should be viewed as a risk factor for NAFLD. Interestingly, weight loss, avoidance of a sedentary life, and effective treatment of sleep apnea syndromes resulted in a significant improvement of liver enzyme levels[41,42].

Clinical recommendation: NAFLD and NASH are diagnoses of exclusion even in obese children with hypertransaminasemia. Other fatty liver causing diseases should be investigated based on the patient's age and clinical features.

Viral infections

Serological markers of hepatitis viruses are part of the first-line etiology investigation panel (Table 2). A positive

history of blood or blood-derivative transfusion, tattooing, ingestion of potentially-contaminated food, and the ethno-geographic origin of the patients give a clue as to a possible infectious etiology. Although immigration and international adoption from endemic areas are well-recognized risk factors, hepatitis B shows an emerging presentation of subclinical chronic hepatitis B in children[43].

In countries where universal vaccination against HBV has been introduced, a significantly-reduced rate of HBV infection in children and mother-to-infant transmission has been observed. However, international adoption and immigration maintain an ample reservoir of HBV infection. Although significantly less infectious than HBV, HCV infection remains a widespread problem in the absence of an effective vaccine. In Western countries, perinatal transmission is currently the primary mode of HCV spread in children, which accounts for approximately 65% of cases[44]. Although hypertransaminasemia may suggest HCV infection, adult[45] and pediatric HCV carriers[46] may have normal or fluctuating aminotransferase levels even in the presence of serious liver damage.

Among the conditions falling within the category of adult cryptogenic hypertransaminasemia is a new entity called "occult" HCV infection. Said entity is characterized by fluctuating serum levels of HCV RNA, while the virus genome and its replicative intermediate RNA-negative strand are detectable in the liver and in peripheral blood mononuclear cells with or without antibodies to HCV[47]. Similarly, "occult" HBV infection has been recognized in patients with cryptogenic hepatitis B surface antigen (HBsAg)-negative chronic liver disease[48]. Intrahepatic replicative activity (HBV covalently closed circular DNA and pregenomic RNA) has recently been described in adults with occult hepatitis B infection. HBV occult infection seems to be relatively frequent in immunized children born to HBsAg-positive mothers. HBsAg negativity is not sufficient for excluding the presence of HBV DNA. These findings emphasize the importance of considering occult HBV infection in hypo-endemic areas[49].

In pediatric patients, hepatitis A virus infection may be followed by up to 1 year intermittent hypertransaminasemia due to relapsing viremia, which does not evolve to chronic liver damage[50]. Other hepatotropic viruses are a frequent cause of liver disease. Epstein-Barr virus and/or cytomegalovirus should be excluded in patients with a history of fever, lymphadenopathy (mainly latero-cervical), pharyngitis, asthenia, and/or hepatosplenomegaly. In patients with paucisymptomatic immunodeficiency, coxsackievirus and adenovirus may be the cause of liver enzyme alteration. Gastrointestinal infection by rotavirus can be accompanied by self-limiting hepatic and non-hepatic transaminase elevation in 20% of infected patients[51].

Currently minor, rather than major, hepatotropic viruses are a common cause of hypertransaminasemia in children in developed countries[12,15,16].

Clinical recommendation: Viral serological markers are part of the first-line investigation panel.

Toxic causes

An accurate medical history is crucial in determining the possible role of toxin-induced hepatotoxicity in children with hypertransaminasemia[52]. In adults, diagnostic scores have been proposed and evaluated to define the association between drugs and liver disease[53,54]. Alcohol abuse can induce liver disease and hypertransaminasemia even in childhood, especially in adolescents[6]. It has been suggested that elevated serum GGT levels, and/or an AST/ALT ratio > 1, and/or an increase in mean corpuscular volume may help in identifying excessive drinking[55]. Carbohydrate deficient transferrin is another tool for identifying unhealthy occult drinking[56]. If medication or alcohol are suspected causes of hypertransaminasemia in an adolescent, aminotransferases should be retested after 6-8 wk of controlled or monitored abstinence[57]. In addition to prescribed or over-the-counter medications and herbal remedies, illegal drugs or substances of abuse should be considered in differential diagnoses and be carefully investigated[58,59].

Autoimmune liver disease

AIH is a progressive inflammatory liver disease without a known etiology, and is characterized histologically by interface hepatitis and serologically by high levels of transaminases, IgG, and positive autoantibodies[60]. The exact prevalence of autoimmune hepatitis is unknown, but it is approximately one in 200000 in the general population of the United States[61]. Sometimes, the histology of autoimmune hepatitis is associated with bile duct injury-determining overlap syndrome or autoimmune sclerosing cholangitis (ASC); this condition is different from primary sclerosing cholangitis, which is characterized by inflammation and fibrosis in the intrahepatic and/or extrahepatic bile load in the absence of interface hepatitis[62,63]. It is important to perform a cholangiography in all children with the histological features of autoimmune hepatitis, as ASC is as prevalent as AIH in childhood, and thus only cholangiography can differentiate between these conditions[64]. There are two types of AIH classified according to antibody profile: type 1 [anti-nuclear antibodies and anti-smooth muscle antibodies (SMA)] and type 2 [anti-liver kidney microsomal antibody (LKM1); and/or antibodies to liver cytosol type 1 (anti-LC1)][65]. Anti-soluble liver antigen (anti-SLA) antibodies can be positive in otherwise autoAb-negative patients. These antibodies cannot be detected by immunofluorescence, and require enzyme-linked immunosorbent assay and immunoassays for identification. Type 2 may tend to be more severe and prevalent in children, adolescents, and young adults than in the older population[60]. The absence of autoantibodies in a child with hypertransaminasemia does not exclude the diagnosis of autoimmune hepatitis. In fact, seronegative but empirically steroid-responsive autoimmune hepatitis has been reported[66-68]. Conversely, the increase in serum gamma-globulins is not universal in AIH. As for Wilson's disease, the diagnosis of autoimmune hepatitis may be difficult because it is not based on specific markers. Consequently, a scoring system

has been devised[69,70] that gives positive predictive values for females, the presence of other autoimmune diseases, hypergammaglobulinemia, and positivity for ANA, SMA, LKM1, LC1 and ASLA. It also gives negative scores for viral markers and a positive history for drugs and alcohol use. A simpler score based on only four items[71] has not yet been fully validated in children. Primary biliary cirrhosis is not observed during childhood, although very rare cases of anti-mitochondrial autoantibody positivity have been reported[72].

AIH (and ASC) may present with only hypertransaminasemia, which can occur in children with apparent good health. It should therefore be investigated with appropriate examinations; if left untreated, cirrhosis may develop.

Clinical recommendation: Autoimmune hepatitis should be rapidly identified in order to administer prompt therapy and avoid cirrhotic evolution. Hypertransaminasemia and hypergammaglobulinemia may be the only findings of seronegative autoimmune hepatitis. It is important to perform a cholangiography in all children with the histological features of autoimmune hepatitis because, in childhood, autoimmune sclerosing cholangitis is as prevalent as autoimmune hepatitis.

Celiac disease and hypertransaminasemia

Celiac disease may be associated with liver involvement in both adults and children. Isolated hypertransaminasemia may be the first manifestation of clinically silent celiac disease[73,74]. It is currently controversial[75] as to whether, in children less than 2 years old, AGA-IgA and IgG should be tested, because of a diagnostic sensitivity higher than that of anti-endomysium antibodies and anti-transglutaminase antibodies at that age. Selective non-responsiveness to HBV immunization in a hypertransaminasemic child may be a clue to undiagnosed celiac disease[76].

So-called "celiac hepatitis" is the most common hepatopathy in celiac patients and is characterized by a moderate increase of transaminase levels usually associated with minimal and non-specific liver lesions of the lobule and portal tracts. A gluten-free diet generally results in normalization of the liver enzymes and repair of histological damage. Due to high disease prevalence, patients with a known diagnosis of celiac disease and persistent hypertransaminasemia should be tested for other possible causes of liver damage before ascribing liver function test abnormalities to celiac disease. Co-existing causes of hepatopathy have been reported[77]. Celiac disease may present as hepatic steatosis in obese children with hypertransaminasemia resistant to weight loss. In such cases, the addition of a gluten-free diet is necessary to resolve their liver abnormalities[34].

Celiac disease can be associated with a variety of autoimmune liver diseases, including AIH, autoimmune cholangitis, and overlap syndromes, with a frequency of AIH peaking at 2.9% in celiac disease children less than 10 years old[74]. Autoimmune hepatic involvement can either precede or follow the diagnosis of celiac disease. It is necessary to diagnose AIH associated with celiac disease promptly, because it is not responsive to a gluten-free diet alone and requires long-term associated immunosuppressive therapy[78].

Wilson's disease and hypertransaminasemia

The prevalence of Wilson's disease is estimated at one in 30000 in most populations (Table 4). The prevalence is as high as one in 10000 in China, Japan, and Sardinia[79]. It may present at any age. Usually, the onset of symptomatic liver disease is at approximately 12 years of age[80]. It is only during adolescence or early adult life that patients usually present with complicating neurological and psychiatric manifestations. The most important laboratory diagnostic clues are hypoceruloplasminemia (< 20 mg/dL in 85%-95% cases), increased free serum copper, increased intrahepatic copper (> 250 μg/g dry weight), and increased basal and post-penicillamine challenge urinary copper excretion[81,82]. These findings are not specific if considered individually, and several conditions may be responsible for false negative and false positive results. Therefore, a score (the "Ferenci score"), which takes into account the Kayser-Fleischer ring, neuropsychiatric symptoms, the occurrence of Coombs negative hemolytic anemia, increased urinary copper, decreased serum ceruloplasmin, increased copper content of hepatocytes, and the presence of causative mutations, has been devised to distinguish between an unlikely/probable/highly-likely diagnosis of Wilson's disease[81]. More recently, a new cut-off value for urinary copper excretion in asymptomatic children with Wilson's disease has been suggested. The new value of 40 μg/24 h replaces the previously-used 100 μg/24 h[83,84]. The post-penicillamine challenge urinary copper estimation has been reported to be poorly sensitive in asymptomatic children[84]. A molecular diagnosis and/or haplotype analysis of the region surrounding ATP7B on chromosome 13 should be considered in children with enigmatic liver disease, especially those with features of NAFLD[84,85]. Wilson's disease-like hypoceruloplasminemic liver disease has been recently described in congenital disorders of glycosylation (CDG) type X[84,86,87]. Non-Wilsonian high urinary copper excretion has been reported in pediatric nodular regenerative hyperplasia[84].

Clinical recommendation: The criteria adopted for the diagnosis of Wilson's disease are non-specific if considered individually. The Ferenci score will distinguish between unlikely, probable, and highly-likely diagnoses of Wilson's disease. New pediatric cut-off values and the real value of post-penicillamine urinary test in asymptomatic cases need careful consideration.

OTHER DISEASES

Disorders, such as inborn errors of metabolism and/or congenital conditions affecting the liver, are much more

Table 4 Clinical and laboratory findings for orienting diagnosis of some genetic metabolic liver diseases

Clinical/laboratory findings	Possible genetic-metabolic cause	Prevalence	Liver involvement
Pancreatic failure, hematological disorders	Shwachman syndrome	1:50000	+++[1]
Asymptomatic, hemolysis	Wilson's disease	1:30000	+++
Previous neonatal cholestasis, hepatomegaly	Alpha 1 antitrypsin deficiency	1:7000	+++
Hypoglycemia, hepatomegaly	Glycogen storage disease (type Ⅰ, Ⅵ and Ⅸ)	From 1:100000 to 1:1000000	+++
Fructose refusal, hepatomegaly	Hereditary fructose intolerance	1:20000	+++
Lethargy, increased serum ammonia levels	Urea cycle defects	1:30000 (all disorders)	++
Lethargy, increased serum ammonia levels	Urea cycle defects	1:30000 (all disorders)	++
Chubby face, fatty liver, specific serum amino acids pattern	Citrin deficiency	1:20000 in East Asia	++
Failure to thrive, lactic acidosis	Mitochondrial diseases	1:8500 (all disorders)	+
Failure to thrive, ketoacidosis, hypoglycemia	Organic acidosis	1:1000 (all disorders)	+
Mild coagulopathy, clinical phenotype	Congenital disorders of glycosylation	From 1:10000 to 1:100000	+
Short stature, female gender, karyotype	Turner syndrome	1:2000	+
Failure to thrive, positive sweat test	Cystic fibrosis	1:2500	+

[1]First 1-2 yr of life; +: Possible; ++: Frequent; +++: Almost always.

common in pediatric patients than in adults (Table 3). These diseases are rare when considered individually, but represent a large group if considered collectively. It is difficult to establish their incidences; in most cases, they are relatively asymptomatic and may therefore remain undiagnosed[88]. Although many etiologies present in the neonatal period with cholestasis or acute illness, several may become manifest only later in infancy or childhood. Extreme care is required to avoid misdiagnosing these cases. This is particularly true if the patients have NAFLD-like symptoms that risk being considered part of the NASH syndrome[38]. Laboratory investigations for genetic metabolic diseases that may often be responsible for a NAFLD-like fatty liver picture should be guided by age and clinical/family history (Table 4). We will comment only on the most relevant metabolic liver diseases and refer the reader to a series of comprehensive reviews for specific information regarding the less common ones[89,90].

Hepatic derangement with severe, but self-limiting, hypertransaminasemia and variable histological patterns may be the sole initial evident manifestation of Shwachman-Diamond syndrome (incidence: 1:50000 worldwide). The mechanisms that can contribute to liver damage in these patients are not known[91]. Citrin deficiency (incidence: 1:20000 in East Asia), a condition now also recognized in Western countries, may present with a pattern of neonatal cholestasis and increased levels of blood citrulline, or NAFLD in children and adolescents[92]. Hereditary fructose intolerance (incidence: 1:20000 worldwide) typically occurs with a pattern of early-onset cholestasis during weaning. However, it may present later in patients who spontaneously follow a low fructose diet because of instinctive fructose refusal/dislike or avoidance. In these cases, medical observation may be dictated by the incidental finding of hypertransaminasemia, hepatomegaly, and/or bright liver by ultrasound observation. Feeding history is crucial for the diagnosis, which is confirmed by molecular analysis of gene mutations[93]. Mitochondrial diseases are often multisystem, but some cases may present with exclusive or prevalent mild liver involvement [*e.g.*, mitochondrial DNA depletion syndrome due to deoxyguanosine kinase (DGUOK, OMIM 251880)]. Some congenital disorders of glycosylation (incidence: 1:50000-1:100000) may present as chronic isolated hypertransaminasemia. Children with clinically asymptomatic, cryptogenic hypertransaminasemia with liver steatosis-fibrosis and mild coagulopathy should be screened for CDG[86,87]. Congenital hepatic fibrosis (CHF, incidence: unknown) is a developmental disorder of the porto-biliary system. It is one of the fibropolycystic diseases, which include Caroli disease, autosomal dominant polycystic kidney disease, and autosomal recessive polycystic kidney disease. Clinically, it is characterized by non- cirrhotic portal hypertension, and rarely complicated by (porto-) pulmonary hypertension and hepatopulmonary syndrome. Hepatocellular function is relatively well-preserved, unless cholangitic episodes are present. Hereditary familial hemochromatosis (incidence: 1:20000 worldwide) most often presents after the transition to maturity, and is well-discussed in studies on adult patients and outside the scope of this article. Alpha 1-antitrypsin deficiency (incidence: 1:7000) presents clinical symptoms only in a minority of affected people. In infancy, the most common presentation is cholestasis. During childhood, it may present with minimally symptomatic disease that becomes significant liver disease only in 10%-15% of patients, often after several years of a near-normal quality of life, and may progress to decompensated liver disease[94]. Glycogenosis types Ⅵ and Ⅸ may be associated with elevated ALT and a soft hepatomegaly that is often not discernible at clinical examination, but without the gross metabolic abnormalities that are typically observed in the other types of glycogenosis. Cystic fibrosis rarely has a prevalent or exclusive hepatic presentation[95]. Nodular regenerative hyperplasia is an infrequently-identified liver disease characterized by non-fibrotic nodular hepatocyte regeneration, secondary portal hypertension, and mild stable abnormalities of liver function tests. In adults, it is usually associated with malignant prothrombotic or rheumatological conditions. These associations are rarely encountered in pediatric practice. The diagnosis is sometimes suggested by minimal histological changes

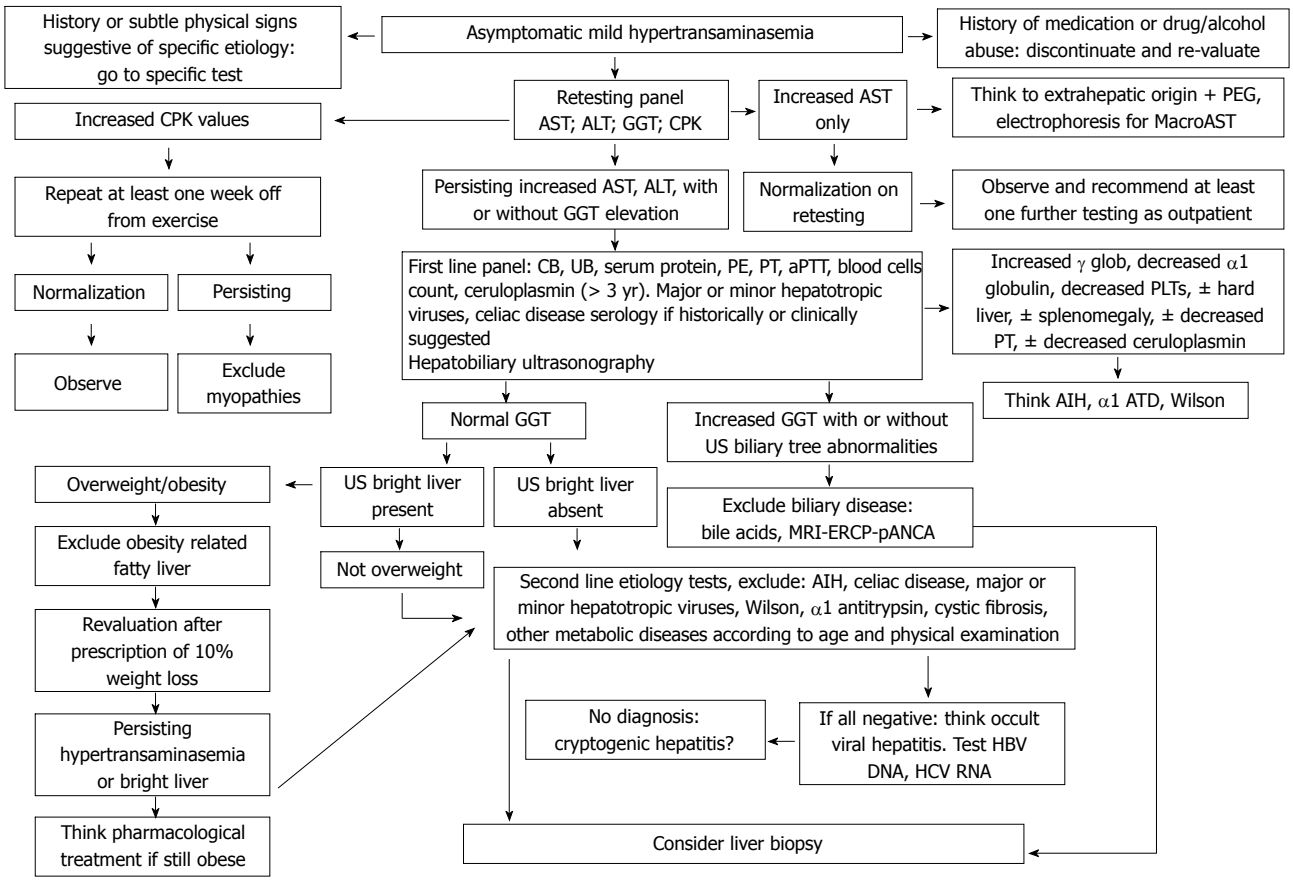

Figure 1 Diagnostic algorithm for the diagnosis of pediatric mild chronic asymptomatic hypertransaminasemia. Modified from the reference of 28. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CB: Conjugated bilirubin; UB: Unconjugated bilirubin; CPK: Creatine kinase; GGT: Gamma-glutamyl transferase; PE: Pulmonary embolism; PEG: Polyethylene glycol; PT: Prothrombin time; PTT: Partial thromboplastin time; US: Ultrasound; MRI: Magnetic resonance imaging; ERCP: Endoscopic retrograde cholangiopancreatography; pANCA: Perinuclear anti-neutrophil cytoplasmic antibodies; HBV: Hepatitis B virus; HCV: Hepatitis C virus; AIH: Autoimmune hepatitis; α1 ATD: α1-antitrypsin deficiency.

(*e.g.*, sinusoidal dilation)[96].

TRANSAMINITIS AND CRYPTOGENIC HEPATITIS

The term "transaminitis" was coined to describe overall liver enzyme leakage without hepatotoxic consequences in adult patients receiving drug therapy of any type. Hypertransaminasemia is due to an unknown effect of the drug (*e.g.*, changes in hepatocellular membranes due to lipid lowering) or underlying conditions (*i.e.*, fatty liver)[97]. Childhood "cryptogenic" hepatitis appears to be a symptomless disease characterized by isolated hypertransaminasemia that onsets during the first 4 years of life, and mild to moderate histologic liver lesions. Although the frequency of spontaneous remissions is low, childhood chronic cryptogenic hepatitis appears, in the short-term, to be a non-progressive disease. This diagnosis can only be attempted once all other known causes of liver disease are excluded[14,15].

DISCUSSION AND CONCLUSION

Given the large number of pediatric conditions that may be responsible for isolated persistent hypertransaminasemia, one can conclude that even asymptomatic children should undergo an intensive liver work-up. Based on the possibility of a fluctuating pattern, it seems reasonable to repeat ALT and AST testing to confirm hypertransaminasemia, rather than embarking on expensive investigations that may prove to be useless. An enzyme panel costs approximately $30, whereas tests to identify only some of the most common causes of elevated liver enzymes (such as serology for hepatitis B and C infection and ultrasonography for NAFLD) cost approximately $400[10]. However, the cost-benefit considerations of a stepwise diagnostic approach *vs* a simultaneous (and timesaving) testing approach in children should not deter from the need to avoid repeated vein punctures, which is often a traumatic experience. As seen in patients with a fever of unknown origin, in asymptomatic children with cryptogenic hypertransaminasemia, ordering investigations as screening procedures in the hope that something abnormal will be identified might have a number of disadvantages. These disadvantages include: possible adverse reactions or complications, loss of the patient's faith in the medical staff, high testing costs, and a soporific effect on the doctor's diagnostic mental activities[98].

The prescription of a "retesting panel", which in-

cludes the determination of GGT and CPK in addition to aminotransferase levels, has the advantage of confirming the persistence of the abnormality, helping to rule out, at least in part, cholestatic hepatopathies and myopathies, and guiding the subsequent diagnostic steps that are shown in Figure 1. Testing serum bile acids and cholangiography are other means to better assess cholestasis. If reassessment of physical and anamnestic findings suggests specific etiologies, these should be checked in the initial enzyme retesting panel (*e.g.*, viral serologies or hepatorenal ultrasonography for viral hepatitis and NAFLD, respectively). In the presence of even subtle symptoms or signs (*e.g.*, jaundice, ascites, pruritus, hepatomegaly, and/or splenomegaly), complete testing to identify the possible cause of liver disease should be included in the initial retesting.

The first line panel in asymptomatic hypertransaminasemic patients should consist of liver ultrasonography, liver function tests, and a number of investigations for the most frequent etiologies. Second and third line investigations are justified either by the inconclusive first line panel or to explore specific plausible conditions. Liver biopsy is part of these panels, but its exact timing and role remains a controversial issue[28,39,99-101]. It has been shown that in those patients with negative etiological investigations, a liver biopsy will most likely not add further useful information[10,15], and considering that a percutaneous liver biopsy samples only 1:50000 of the liver, sampling error is an obvious limitation which can lead to misdiagnosis and staging inaccuracies[102]. The competence of the pediatric liver disease pathologist is paramount. Steatosis of the liver in a non-obese individual may suggest a metabolic/genetic hepatopathy[14,38].

In conclusion, here we provide an overview of pediatric persistent hypertransaminasemia and list a series of metabolic, genetic, gastrointestinal, and extrahepatic causes that should be taken into account in clinical practice. The number of these etiologies constitutes a wider field of what one usually considers in adulthood. Importantly, information derived from the combination of the patient's history, physical examination, and basic laboratory data are necessary to reach a timely and correct diagnosis. We also provide a stepwise approach that should always be guided by clinical scenarios.

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REFERENCES

1 **Rook M**, Rosenthal P. Caring for adults with pediatric liver disease. *Curr Gastroenterol Rep* 2009; **11**: 83-89 [PMID: 19166664 DOI: 10.1007/s11894-009-0013-7]
2 **Riley MR**, Bass NM, Rosenthal P, Merriman RB. Underdiagnosis of pediatric obesity and underscreening for fatty liver disease and metabolic syndrome by pediatricians and pediatric subspecialists. *J Pediatr* 2005; **147**: 839-842 [PMID: 16356443 DOI: 10.1016/j.jpeds.2005.07.020]
3 **Franzese A**, Vajro P, Argenziano A, Puzziello A, Iannucci MP, Saviano MC, Brunetti F, Rubino A. Liver involvement in obese children. Ultrasonography and liver enzyme levels at diagnosis and during follow-up in an Italian population. *Dig Dis Sci* 1997; **42**: 1428-1432 [PMID: 9246041]
4 **Morisco F**, Pagliaro L, Caporaso N, Bianco E, Sagliocca L, Fargion S, Smedile A, Salvagnini M, Mele A. Consensus recommendations for managing asymptomatic persistent non-virus non-alcohol related elevation of aminotransferase levels: suggestions for diagnostic procedures and monitoring. *Dig Liver Dis* 2008; **40**: 585-598 [PMID: 18395501 DOI: 10.1016/j.dld.2008.02.003]
5 **Pratt DS**, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N Engl J Med* 2000; **342**: 1266-1271 [PMID: 10781624 DOI: 10.1056/NEJM200004273421707]
6 **Clark DB**, Lynch KG, Donovan JE, Block GD. Health problems in adolescents with alcohol use disorders: self-report, liver injury, and physical examination findings and correlates. *Alcohol Clin Exp Res* 2001; **25**: 1350-1359 [PMID: 11584156 DOI: 10.1111/j.1530-0277.2001.tb02358.x]
7 **Cobbold JF**, Anstee QM, Thomas HC. Investigating mildly abnormal serum aminotransferase values. *BMJ* 2010; **341**: c4039 [PMID: 20675393 DOI: 10.1136/bmj.c4039]
8 **Giboney PT**. Mildly elevated liver transaminase levels in the asymptomatic patient. *Am Fam Physician* 2005; **71**: 1105-1110 [PMID: 15791889]
9 **Giannini EG**, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. *CMAJ* 2005; **172**: 367-379 [PMID: 15684121 DOI: 10.1503/cmaj.1040752]
10 **Green RM**, Flamm S. AGA technical review on the evaluation of liver chemistry tests. *Gastroenterology* 2002; **123**: 1367-1384 [PMID: 12360498 DOI: 10.1053/gast.2002.36061]
11 **Krier M**, Ahmed A. The asymptomatic outpatient with abnormal liver function tests. *Clin Liver Dis* 2009; **13**: 167-177 [PMID: 19442912 DOI: 10.1016/j.cld.2009.02.001]
12 **Iorio R**, Sepe A, Giannattasio A, Cirillo F, Vegnente A. Hypertransaminasemia in childhood as a marker of genetic liver disorders. *J Gastroenterol* 2005; **40**: 820-826 [PMID: 16143887 DOI: 10.1007/s00535-005-1635-7]
13 **Matsui A**. Hypertransaminasemia: the end of a thread. *J Gastroenterol* 2005; **40**: 859-860 [PMID: 16143897 DOI: 10.1007/s00535-005-1675-z]
14 **Zancan L**, Bettiol T, Rini A, Giacchino R, Gandullia P, Vajro P, Marcellini M, Musumeci S, Di Leo G, Balli F, Bragetti P, Calacoci M, Barbera C. Chronic idiopathic hypertransaminasemia. *Minerva Pediatr* 1996; **48**: 209-216 [PMID: 8926957]
15 **Bugeac N**, Pacht A, Mandel H, Iancu T, Tamir A, Srugo I, Shaoul R. The significance of isolated elevation of serum aminotransferases in infants and young children. *Arch Dis Child* 2007; **92**: 1109-1112 [PMID: 17652319 DOI: 10.1136/adc.2007.121194]
16 **Nobili V**, Reale A, Alisi A, Morino G, Trenta I, Pisani M, Marcellini M, Raucci U. Elevated serum ALT in children presenting to the emergency unit: Relationship with NAFLD. *Dig Liver Dis* 2009; **41**: 749-752 [PMID: 19362523 DOI: 10.1016/j.dld.2009.02.048]
17 **Kohli R**, Harris DC, Whitington PF. Relative elevations of serum alanine and aspartate aminotransferase in muscular dystrophy. *J Pediatr Gastroenterol Nutr* 2005; **41**: 121-124 [PMID: 15990642 DOI: 10.1097/01.WNO.0000161657.98895.97]
18 **Piton A**, Poynard T, Imbert-Bismut F, Khalil L, Delattre J, Pelissier E, Sansonetti N, Opolon P. Factors associated with serum alanine transaminase activity in healthy subjects: consequences for the definition of normal values, for selection of blood donors, and for patients with chronic hepatitis C. MULTIVIRC Group. *Hepatology* 1998; **27**: 1213-1219 [PMID: 9581673 DOI: 10.1002/hep.510270505]
19 **Prati D**, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, Vianello L, Zanuso F, Mozzi F, Milani S, Conte D, Colombo M, Sirchia G. Updated definitions of healthy

ranges for serum alanine aminotransferase levels. *Ann Intern Med* 2002; **137**: 1-10 [PMID: 12093239]
20 **England K**, Thorne C, Pembrey L, Tovo PA, Newell ML. Age- and sex-related reference ranges of alanine aminotransferase levels in children: European paediatric HCV network. *J Pediatr Gastroenterol Nutr* 2009; **49**: 71-77 [PMID: 19465871 DOI: 10.1097/MPG.0b013e31818fc63b]
21 **Schwimmer JB**, Dunn W, Norman GJ, Pardee PE, Middleton MS, Kerkar N, Sirlin CB. SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. *Gastroenterology* 2010; **138**: 1357-1364, 1364.e1-2 [PMID: 20064512 DOI: 10.1053/j.gastro.2009.12.052]
22 **Veropalumbo C**, Del Giudice E, Esposito G, Maddaluno S, Ruggiero L, Vajro P. Aminotransferases and muscular diseases: a disregarded lesson. Case reports and review of the literature. *J Paediatr Child Health* 2012; **48**: 886-890 [PMID: 20500440 DOI: 10.1111/j.1440-1754.2010.01730.x]
23 **Lazo M**, Selvin E, Clark JM. Brief communication: clinical implications of short-term variability in liver function test results. *Ann Intern Med* 2008; **148**: 348-352 [PMID: 18316753]
24 **Senadhi V**. A paradigm shift in the outpatient approach to liver function tests. *South Med J* 2011; **104**: 521-525 [PMID: 21886053 DOI: 10.1097/SMJ.0b013e31821e8ff5]
25 **Pettersson J**, Hindorf U, Persson P, Bengtsson T, Malmqvist U, Werkström V, Ekelund M. Muscular exercise can cause highly pathological liver function tests in healthy men. *Br J Clin Pharmacol* 2008; **65**: 253-259 [PMID: 17764474 DOI: 10.1111/j.1365-2125.2007.03001.x]
26 **Bruno C**, Sotgia F, Gazzerro E, Minetti C, Lisanti MP. Caveolinopathies. In: Pagon RA, Bird TD, Dolan CR, Stephens K, Adam MP, editors. GeneReviews™ [Internet]. Seattle (WA): University of Washington, 1993-2007 [PMID: 20301559]
27 **Musana KA**, Yale SH, Abdulkarim AS. Tests of liver injury. *Clin Med Res* 2004; **2**: 129-131 [PMID: 15931347 DOI: 10.3121/cmr.2.2.129]
28 **Vajro P**, Di Cosmo N, Capuano G. Approach to the asymptomatic child with protracted hypertransaminasemia. In: Guandalini S. Essential Pediatric Gastroenterology, Hepatology and Nutrition. New York: McGraw-Hill, 2005: 335-344
29 **Caropreso M**, Fortunato G, Lenta S, Palmieri D, Esposito M, Vitale DF, Iorio R, Vajro P. Prevalence and long-term course of macro-aspartate aminotransferase in children. *J Pediatr* 2009; **154**: 744-748 [PMID: 19111320]
30 **Roberts EA**. Pediatric nonalcoholic fatty liver disease (NAFLD): a "growing" problem? *J Hepatol* 2007; **46**: 1133-1142 [PMID: 17445934 DOI: 10.1016/j.jhep.2007.03.003]
31 **Alisi A**, Manco M, Vania A, Nobili V. Pediatric nonalcoholic fatty liver disease in 2009. *J Pediatr* 2009; **155**: 469-474 [PMID: 19772998 DOI: 10.1016/j.jpeds.2009.06.014]
32 **Fishbein MH**, Miner M, Mogren C, Chalekson J. The spectrum of fatty liver in obese children and the relationship of serum aminotransferases to severity of steatosis. *J Pediatr Gastroenterol Nutr* 2003; **36**: 54-61 [PMID: 12499997 DOI: 10.1097/00005176-200301000-00012]
33 **Patton HM**, Lavine JE, Van Natta ML, Schwimmer JB, Kleiner D, Molleston J. Clinical correlates of histopathology in pediatric nonalcoholic steatohepatitis. *Gastroenterology* 2008; **135**: 1961-1971.e2 [PMID: 19013463 DOI: 10.1053/j.gastro.2008.08.050]
34 **Franzese A**, Iannucci MP, Valerio G, Ciccimarra E, Spaziano M, Mandato C, Vajro P. Atypical celiac disease presenting as obesity-related liver dysfunction. *J Pediatr Gastroenterol Nutr* 2001; **33**: 329-332 [PMID: 11593131 DOI: 10.1097/00005176-200109000-00019]
35 **Tucker E**, Rostami K, Prabhakaran S, Al Dulaimi D. Patients with coeliac disease are increasingly overweight or obese on presentation. *J Gastrointestin Liver Dis* 2012; **21**: 11-15 [PMID: 22457854]
36 **Spindelboeck W**, Deutschmann A, Lackner K, Weitzer C, Erwa W, Hauer A Ch, Ferenci P, Deutsch J. Obesity and Wilson disease in Childhood and adolescence. *J Pediatr Gastroenterol Nutr* 2009; **48** Suppl 3: E63
37 **Adams LA**, Lindor KD, Angulo P. The prevalence of autoantibodies and autoimmune hepatitis in patients with nonalcoholic Fatty liver disease. *Am J Gastroenterol* 2004; **99**: 1316-1320 [PMID: 15233671 DOI: 10.1111/j.1572-0241.2004.30444.x]
38 **Cassiman D**, Jaeken J. NASH may be trash. *Gut* 2008; **57**: 141-144 [PMID: 18192446 DOI: 10.1136/gut.2007.123240]
39 **Vajro P**, Lenta S, Socha P, Dhawan A, McKiernan P, Baumann U, Durmaz O, Lacaille F, McLin V, Nobili V. Diagnosis of nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN Hepatology Committee. *J Pediatr Gastroenterol Nutr* 2012; **54**: 700-713 [PMID: 22395188 DOI: 10.1097/MPG.0b013e318252a13f]
40 **Manco M**, Bedogni G, Marcellini M, Devito R, Ciampalini P, Sartorelli MR, Comparcola D, Piemonte F, Nobili V. Waist circumference correlates with liver fibrosis in children with non-alcoholic steatohepatitis. *Gut* 2008; **57**: 1283-1287 [PMID: 18218674 DOI: 10.1136/gut.2007.142919]
41 **Verhulst SL**, Jacobs S, Aerts L, Schrauwen N, Haentjens D, Rooman RP, Gaal LV, De Backer WA, Desager KN. Sleep-disordered breathing: a new risk factor of suspected fatty liver disease in overweight children and adolescents? *Sleep Breath* 2009; **13**: 207-210 [PMID: 19002513 DOI: 10.1007/s11325-008-0231-5]
42 **Kheirandish-Gozal L**, Sans Capdevila O, Kheirandish E, Gozal D. Elevated serum aminotransferase levels in children at risk for obstructive sleep apnea. *Chest* 2008; **133**: 92-99 [PMID: 18187742 DOI: 10.1378/chest.07-0773]
43 **Sciveres M**, Maggiore G. Hepatitis B "by proxy": an emerging presentation of chronic hepatitis B in children. *J Pediatr Gastroenterol Nutr* 2007; **44**: 268-269 [PMID: 17255843 DOI: 10.1097/MPG.0b013e31802c69bb]
44 **Stephenne X**, Sokal EM. Hepatitis C in children and adolescents: mode of acquisition, natural history and treatment. *Acta Gastroenterol Belg* 2002; **65**: 95-98 [PMID: 12148447]
45 **Puoti C**, Bellis L, Guarisco R, Dell' Unto O, Spilabotti L, Costanza OM. HCV carriers with normal alanine aminotransferase levels: healthy persons or severely ill patients? Dealing with an everyday clinical problem. *Eur J Intern Med* 2010; **21**: 57-61 [PMID: 20206870 DOI: 10.1016/j.ejim.2009.12.006]
46 **Iorio R**, Giannattasio A, Sepe A, Terracciano LM, Vecchione R, Vegnente A. Chronic hepatitis C in childhood: an 18-year experience. *Clin Infect Dis* 2005; **41**: 1431-1437 [PMID: 16231253 DOI: 10.1086/497141]
47 **Pham TN**, Coffin CS, Michalak TI. Occult hepatitis C virus infection: what does it mean? *Liver Int* 2010; **30**: 502-511 [PMID: 20070513 DOI: 10.1111/j.1478-3231.2009.02193.x]
48 **Fang Y**, Shang QL, Liu JY, Li D, Xu WZ, Teng X, Zhao HW, Fu LJ, Zhang FM, Gu HX. Prevalence of occult hepatitis B virus infection among hepatopathy patients and healthy people in China. *J Infect* 2009; **58**: 383-388 [PMID: 19329189 DOI: 10.1016/j.jinf.2009.02.013]
49 **Shahmoradi S**, Yahyapour Y, Mahmoodi M, Alavian SM, Fazeli Z, Jazayeri SM. High prevalence of occult hepatitis B virus infection in children born to HBsAg-positive mothers despite prophylaxis with hepatitis B vaccination and HBIG. *J Hepatol* 2012; **57**: 515-521 [PMID: 22617152 DOI: 10.1016/j.jhep.2012.04.021]
50 **Arslan S**, Caksen H, Oner AF, Odabaş D, Rastgeldi L. Relapsing hepatitis A in children: report of two cases. *Acta Paediatr Taiwan* 2002; **43**: 358-360 [PMID: 12632793]
51 **Teitelbaum JE**, Daghistani R. Rotavirus causes hepatic transaminase elevation. *Dig Dis Sci* 2007; **52**: 3396-3398 [PMID: 17431773 DOI: 10.1007/s10620-007-9743-2]
52 **Navarro VJ**, Senior JR. Drug-related hepatotoxicity. *N Engl J Med* 2006; **354**: 731-739 [PMID: 16481640 DOI: 10.1056/NEJMra052270]

53 **Maria VA**, Victorino RM. Development and validation of a clinical scale for the diagnosis of drug-induced hepatitis. *Hepatology* 1997; **26**: 664-669 [PMID: 9303497 DOI: 10.1002/hep.510260319]

54 **Aithal GP**, Rawlins MD, Day CP. Clinical diagnostic scale: a useful tool in the evaluation of suspected hepatotoxic adverse drug reactions. *J Hepatol* 2000; **33**: 949-952 [PMID: 11131457 DOI: 10.1016/S0168-8278(00)80127-0]

55 **Liangpunsakul S**, Qi R, Crabb DW, Witzmann F. Relationship between alcohol drinking and aspartate aminotransferase: alanine aminotransferase (AST: ALT) ratio, mean corpuscular volume (MCV), gamma-glutamyl transpeptidase (GGT), and apolipoprotein A1 and B in the U.S. population. *J Stud Alcohol Drugs* 2010; **71**: 249-252 [PMID: 20230722]

56 **Conigrave KM**, Davies P, Haber P, Whitfield JB. Traditional markers of excessive alcohol use. *Addiction* 2003; **98** Suppl 2: 31-43 [PMID: 14984240 DOI: 10.1046/j.1359-6357.2003.00581.x]

57 **Aragon G**, Younossi ZM. When and how to evaluate mildly elevated liver enzymes in apparently healthy patients. *Cleve Clin J Med* 2010; **77**: 195-204 [PMID: 20200170 DOI: 10.3949/ccjm.77a.09064]

58 **Fidler H**, Dhillon A, Gertner D, Burroughs A. Chronic ecstasy (3,4-methylenedioxymetamphetamine) abuse: a recurrent and unpredictable cause of severe acute hepatitis. *J Hepatol* 1996; **25**: 563-566 [PMID: 8912157 DOI: 10.1016/S0168-8278(96)80217-0]

59 **Devlin RJ**, Henry JA. Clinical review: Major consequences of illicit drug consumption. *Crit Care* 2008; **12**: 202 [PMID: 18279535 DOI: 10.1186/cc6166]

60 **Mieli-Vergani G**, Vergani D. Autoimmune hepatitis. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 320-329 [PMID: 21537351 DOI: 10.1038/nrgastro.2011.69]

61 **Boberg KM**. Prevalence and epidemiology of autoimmune hepatitis. *Clin Liver Dis* 2002; **6**: 635-647 [PMID: 12362572 DOI: 10.1016/S1089-3261(02)00021-1]

62 **Maggiore G**, Riva S, Sciveres M. Autoimmune diseases of the liver and biliary tract and overlap syndromes in childhood. *Minerva Gastroenterol Dietol* 2009; **55**: 53-70 [PMID: 19212308]

63 **Kerkar N**, Miloh T. Sclerosing cholangitis: pediatric perspective. *Curr Gastroenterol Rep* 2010; **12**: 195-202 [PMID: 20425475 DOI: 10.1007/s11894-010-0104-5]

64 **Mieli-Vergani G**, Vergani D. Autoimmune hepatitis in children: what is different from adult AIH? *Semin Liver Dis* 2009; **29**: 297-306 [PMID: 19676002 DOI: 10.1055/s-0029-1233529]

65 **Bridoux-Henno L**, Maggiore G, Johanet C, Fabre M, Vajro P, Dommergues JP, Reinert P, Bernard O. Features and outcome of autoimmune hepatitis type 2 presenting with isolated positivity for anti-liver cytosol antibody. *Clin Gastroenterol Hepatol* 2004; **2**: 825-830 [PMID: 15354284 DOI: 10.1016/S1542-3565(04)00354-4]

66 **Maggiore G**, Roux K, Johanet C, Fabre M, Sciveres M, Riva S, Fournier-Favre S, Bernard O. Seronegative autoimmune hepatitis in childhood. *J Pediatr Gastroenterol Nutr* 2006; **42**: E5-E6

67 **Gassert DJ**, Garcia H, Tanaka K, Reinus JF. Corticosteroid-responsive cryptogenic chronic hepatitis: evidence for seronegative autoimmune hepatitis. *Dig Dis Sci* 2007; **52**: 2433-2437 [PMID: 17429719 DOI: 10.1007/s10620-006-9665-4]

68 **Heringlake S**, Schütte A, Flemming P, Schmiegel W, Manns MP, Tillmann HL. Presumed cryptogenic liver disease in Germany: High prevalence of autoantibody-negative autoimmune hepatitis, low prevalence of NASH, no evidence for occult viral etiology. *Z Gastroenterol* 2009; **47**: 417-423 [PMID: 19418409 DOI: 10.1055/s-0028-1109146]

69 **Vergani D**, Alvarez F, Bianchi FB, Cançado EL, Mackay IR, Manns MP, Nishioka M, Penner E. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. *J Hepatol* 2004; **41**: 677-683 [PMID: 15464251 DOI: 10.1016/j.jhep.2004.08.002]

70 **Ebbeson RL**, Schreiber RA. Diagnosing autoimmune hepatitis in children: is the International Autoimmune Hepatitis Group scoring system useful? *Clin Gastroenterol Hepatol* 2004; **2**: 935-940 [PMID: 15476158 DOI: 10.1016/S1542-3565(04)00396-9]

71 **Hennes EM**, Zeniya M, Czaja AJ, Parés A, Dalekos GN, Krawitt EL, Bittencourt PL, Porta G, Boberg KM, Hofer H, Bianchi FB, Shibata M, Schramm C, Eisenmann de Torres B, Galle PR, McFarlane I, Dienes HP, Lohse AW. Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology* 2008; **48**: 169-176 [PMID: 18537184 DOI: 10.1002/hep.22322]

72 **Gregorio GV**, Portmann B, Mowat AP, Vergani D, Mieli-Vergani G. A 12-year-old girl with antimitochondrial antibody-positive autoimmune hepatitis. *J Hepatol* 1997; **27**: 751-754 [PMID: 9365052 DOI: 10.1016/S0168-8278(97)80093-1]

73 **Vajro P**, Fontanella A, Mayer M, De Vincenzo A, Terracciano LM, D'Armiento M, Vecchione R. Elevated serum aminotransferase activity as an early manifestation of gluten-sensitive enteropathy. *J Pediatr* 1993; **122**: 416-419 [PMID: 8441098 DOI: 10.1016/S0022-3476(05)83430-4]

74 **Vajro P**, Paolella G, Pisano P, Maggiore G. Hypertransaminasemia and coeliac disease. *Aliment Pharmacol Ther* 2012; **35**: 202-203; author reply 203-204 [PMID: 22150542 DOI: 10.1111/j.1365-2036.2011.04895.x]

75 **Foucher B**, Johanet C, Jégo-Desplat S, Sanmarco M, Dubucquoi S, Fily-Nalewajk S, Olsson NO, Lakomy D, Escande A, Chrétien P, Fortenfant F, Chevailler A, André C, Goetz J, Humbel RL, Monier JC, Sibilia J, Taillefer MF, Abreu I, Fabien N. Are immunoglobulin A anti-gliadin antibodies helpful in diagnosing coeliac disease in children younger than 2 years? *J Pediatr Gastroenterol Nutr* 2012; **54**: 110-112 [PMID: 21857243 DOI: 10.1097/MPG.0b013e31823255c9]

76 **Vajro P**, Paolella G, Nobili V. Children unresponsive to hepatitis B virus vaccination also need celiac disease testing. *J Pediatr Gastroenterol Nutr* 2012; **55**: e131 [PMID: 22576672 DOI: 10.1097/MPG.0b013e31825d4ac9]

77 **Di Biase AR**, Colecchia A, Scaioli E, Berri R, Viola L, Vestito A, Balli F, Festi D. Autoimmune liver diseases in a paediatric population with coeliac disease - a 10-year single-centre experience. *Aliment Pharmacol Ther* 2010; **31**: 253-260 [PMID: 19878151 DOI: 10.1111/j.1365-2036.2009.04186.x]

78 **Caprai S**, Vajro P, Ventura A, Sciveres M, Maggiore G. Autoimmune liver disease associated with celiac disease in childhood: a multicenter study. *Clin Gastroenterol Hepatol* 2008; **6**: 803-806 [PMID: 18258488 DOI: 10.1016/j.cgh.2007.12.002]

79 **Cox DW**, Roberts E. Wilson Disease. In: Pagon RA, Bird TD, Dolan CR, Stephens K, Adam MP, editors. GeneReviews™ [Internet]. Seattle (WA): University of Washington, 1993-1999 [PMID: 20301685]

80 **Dhawan A**, Taylor RM, Cheeseman P, De Silva P, Katsiyiannakis L, Mieli-Vergani G. Wilson's disease in children: 37-year experience and revised King's score for liver transplantation. *Liver Transpl* 2005; **11**: 441-448 [PMID: 15776453 DOI: 10.1002/lt.20352]

81 **Ferenci P**, Caca K, Loudianos G, Mieli-Vergani G, Tanner S, Sternlieb I, Schilsky M, Cox D, Berr F. Diagnosis and phenotypic classification of Wilson disease. *Liver Int* 2003; **23**: 139-142 [PMID: 12955875 DOI: 10.1034/j.1600-0676.2003.00824.x]

82 **Vajro P**, Nicastro E. Wilson's disease. In: Jara P. International Hepatology Updates: Liver diseases in children. Barcelona: Edition Permanyer, 2009: 73-90

83 **Nicastro E**, Ranucci G, Vajro P, Vegnente A, Iorio R. Re-evaluation of the diagnostic criteria for Wilson disease in children with mild liver disease. *Hepatology* 2010; **52**: 1948-1956 [PMID: 20967755 DOI: 10.1002/hep.23910]

84 **European Association for Study of Liver.** EASL Clinical Practice Guidelines: Wilson's disease. *J Hepatol* 2012; **56**: 671-685 [PMID: 22340672 DOI:10.1016/j.jhep.2011.11.007]

85 **Caprai S**, Loudianos G, Massei F, Gori L, Lovicu M, Maggiore G. Direct diagnosis of Wilson disease by molecular

genetics. *J Pediatr* 2006; **148**: 138-140 [PMID: 16423615 DOI: 10.1016/j.jpeds.2005.07.036]
86 **Mandato C**, Brive L, Miura Y, Davis JA, Di Cosmo N, Lucariello S, Pagliardini S, Seo NS, Parenti G, Vecchione R, Freeze HH, Vajro P. Cryptogenic liver disease in four children: a novel congenital disorder of glycosylation. *Pediatr Res* 2006; **59**: 293-298 [PMID: 16439595 DOI: 10.1203/01.pdr.0000196378.30165.26]
87 **Calvo PL**, Pagliardini S, Baldi M, Pucci A, Sturiale L, Garozzo D, Vinciguerra T, Barbera C, Jaeken J. Long-standing mild hypertransaminasaemia caused by congenital disorder of glycosylation (CDG) type IIx. *J Inherit Metab Dis* 2008; **31** Suppl 2: S437-S440 [PMID: 19067230 DOI: 10.1007/s10545-008-1004-9]
88 **Scriver CR**, Beaudet AL, Sly WS, Valle D, Childs B. The metabolic and molecular Bases of inherited disease. 9th ed. New York, 2005
89 **Kelly D**. Diseases of the liver and biliary system in children. 3rd ed. Oxford, 2008
90 **Suchy FJ**, Sokol RJ, Balistreri W. Liver disease in children. 3rd ed. Oxford, 2007
91 **Toiviainen-Salo S**, Durie PR, Numminen K, Heikkilä P, Marttinen E, Savilahti E, Mäkitie O. The natural history of Shwachman-Diamond syndrome-associated liver disease from childhood to adulthood. *J Pediatr* 2009; **155**: 807-811.e2 [PMID: 19683257 DOI: 10.1016/j.jpeds.2009.06.047]
92 **Vajro P**, Veropalumbo C. Citrin deficiency: learn more, and don't forget to add it to the list of neonatal cholestasis and the NASH trash bin. *J Pediatr Gastroenterol Nutr* 2010; **50**: 578-579 [PMID: 20479677 DOI: 10.1097/MPG.0b013e3181dee0e3]
93 **Wong D**. Hereditary fructose intolerance. *Mol Genet Metab* 2005; **85**: 165-167 [PMID: 16086449]
94 **Mailliard ME**, Gollan JL. Metabolic liver disease in the young adult. *Best Pract Res Clin Gastroenterol* 2003; **17**: 307-322 [PMID: 12676121 DOI: 10.1016/S1521-6918(02)00148-8]
95 **Resti M**, Adami Lami C, Tucci F, Mannelli F, Rossi ME, Azzari C, Vierucci A. False diagnosis of non-A/non-B hepatitis hiding two cases of cystic fibrosis. *Eur J Pediatr* 1990; **150**: 97-99 [PMID: 2126237 DOI: 10.1007/BF02072047]
96 **Fabre A**, Audet P, Vilgrain V, Nguyen BN, Valla D, Belghiti J, Degott C. Histologic scoring of liver biopsy in focal nodular hyperplasia with atypical presentation. *Hepatology* 2002; **35**: 414-420 [PMID: 11826417 DOI: 10.1053/jhep.2002.31103]
97 **Dujovne CA**. Side effects of statins: hepatitis versus "transaminitis"-myositis versus "CPKitis". *Am J Cardiol* 2002; **89**: 1411-1413 [PMID: 12062737 DOI: 10.1016/S0002-9149(02)02356-1]
98 **de Kleijn EM**, van Lier HJ, van der Meer JW. Fever of unknown origin (FUO). II. Diagnostic procedures in a prospective multicenter study of 167 patients. The Netherlands FUO Study Group. *Medicine* (Baltimore) 1997; **76**: 401-414 [PMID: 9413426 DOI: 10.1097/00005792-199711000-00003]
99 **Iorio R**, Verrico A, Giannattasio A. Is liver biopsy mandatory in children with chronic hepatitis C? *World J Gastroenterol* 2007; **13**: 4025-4026 [PMID: 17663524]
100 **Kumari N,** Kathuria R, Srivastav A, Krishnani N, Poddar U, Yachha SK. Significance of histopathological features in differentiating autoimmune liver disease from nonautoimmune chronic liver disease in children. *Eur J Gastroenterol Hepatol* 2013; **25**: 333-337 [PMID: 23085577 DOI: 10.1097/MEG.0b013e32835a68a1]
101 **Ovchinsky N**, Moreira RK, Lefkowitch JH, Lavine JE. Liver biopsy in modern clinical practice: a pediatric point-of-view. *Adv Anat Pathol* 2012; **19**: 250-262 [PMID: 22692288 DOI: 10.1097/PAP.0b013e31825c6a20]
102 **Ratziu V**, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F, Poynard T. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* 2005; **128**: 1898-1906 [PMID: 15940625 DOI: 10.1053/j.gastro.2005.03.084]

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ORIGINAL ARTICLE

Efficacy and safety of over-the-scope clip: Including complications after endoscopic submucosal dissection

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Abstract

AIM: To retrospectively review the results of over-the-scope clip (OTSC) use in our hospital and to examine the feasibility of using the OTSC to treat perforations after endoscopic submucosal dissection (ESD).

METHODS: We enrolled 23 patients who presented with gastrointestinal (GI) bleeding, fistulae and perforations and were treated with OTSCs (Ovesco Endoscopy GmbH, Tuebingen, Germany) between November 2011 and September 2012. Maximum lesion size was defined as lesion diameter. The number of OTSCs to be used per patient was not decided until the lesion was completely closed. We used a twin grasper (Ovesco Endoscopy GmbH, Tuebingen, Germany) as a grasping device for all the patients. A 9 mm OTSC was chosen for use in the esophagus and colon, and a 10 mm device was used for the stomach, duodenum and rectum. The overall success rate and complications were evaluated, with a particular emphasis on patients who had undergone ESD due to adenocarcinoma. In technical successful cases we included not only complete closing by using OTSCs, but also partial closing where complete closure with OTSCs is almost difficult. In overall clinical successful cases we included only complete closing by using only OTSCs perfectly. All the OTSCs were placed by 2 experienced endoscopists. The sites closed after ESD included not only the perforation site but also all defective ulcers sites.

RESULTS: A total of 23 patients [mean age 77 years (range 64-98 years)] underwent OTSC placement during the study period. The indications for OTSC placement were GI bleeding (n = 9), perforation (n = 10), fistula (n = 4) and the prevention of post-ESD duodenal artificial ulcer perforation (n = 1). One patient had a perforation caused by a glycerin enema, after which a fistula formed. Lesion closure using the OTSC alone was successful in 19 out of 23 patients, and overall success rate was 82.6%. A large lesion size (greater than 20 mm) and a delayed diagnosis (more than 1 wk) were the major contributing factors for the overall unsuccessful clinical cases. The location of the unsuccessful lesion was in the stomach. The median operation time in the successful cases was 18 min, and the average observation time was 67 d. During the observation period, none of the patients experienced any complications associated with OTSC placement. In addition, we successfully used the OTSC to close the perforation site after ESD in 6 patients. This was a single-center, retrospective study with a small sample size.

CONCLUSION: The OTSC is effective for treating GI bleeding, fistulae as well as perforations, and the OTSC technique proofed effective treatment for perforation after ESD.

Key words: Over-the-scope clip; Gastrointestinal bleed-

ing; Endoscopic submucosal dissection complications; Gastrointestinal fistulae; Gastrointestinal perforation

Core tip: We have reported our experiences with the over-the-scope clip (OTSC) and the outcomes of several cases in Japan. The aims of the present study were to retrospectively review the results of OTSC use in our hospital and to examine the feasibility of using the OTSC to completely close perforations after endoscopic submucosal dissection for early gastrointestinal cancers.

Nishiyama N, Mori H, Kobara H, Rafiq K, Fujihara S, Kobayashi M, Oryu M, Masaki T. Efficacy and safety of over-the-scope clip: Including complications after endoscopic submucosal dissection. *World J Gastroenterol* 2013; 19(18): 2752-2760 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i18/2752.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i18.2752

INTRODUCTION

Historically, the standard treatment for gastrointestinal (GI) perforations has been surgery. Recent, invasive endoscopic treatments, such as endoscopic submucosal dissection (ESD) and natural orifice transluminal endoscopic surgery (NOTES), have provided alternative approaches to surgery. Our recent attempts to develop a new device, such as a full-thickness suturing device, constitute progress in the development of NOTES[1-4]. In addition, several devices are currently available for endoscopic management[5,6]. However, there is little convenient, safety and perfect device for complication of endoscopic treatment[7,8]. Since its introduction, the over-the-scope clip (OTSC) (Ovesco Endoscopy GmbH, Tuebingen, Germany) has been used to treat GI bleeding, fistulae and perforations in the United States and several European countries. Reports describing the use and value of OTSCs have primarily consisted of animal studies and clinical cases[9-14]. Retrospective studies have demonstrated the feasibility and the preliminary safety of the OTSC for the treatment of GI bleeding and fistulae, as well as for the closure of acute GI perforations[15,16]. The OTSC was approved by the Japanese Drug Administration and was made commercially available in November 2011. We have reported on our experiences with the OTSC and the outcomes of several cases involving its use since this device became available in Japan. Here, we retrospectively report the results of using the OTSC in our hospital. We also describe the potential use of the OTSC to completely close perforations after ESD for early GI cancers.

MATERIALS AND METHODS

We retrospectively analyzed our database of all 23 patients who underwent OTSC placement (Ovesco Endoscopy GmbH, Tuebingen, Germany) in our hospital from November 2011 to September 2012, as summarized in Table 1. The indications for OTSC placement were GI bleeding, perforations, fistulae and the prevention of post-ESD duodenal artificial ulcer perforation. ESD were performed because of dissection of adenocarcinoma. Maximum lesion size was defined as lesion diameter, not lesion surface area. The number of OTSCs to be used per patient was not decided until the lesion was completely closed. We used a twin grasper (Ovesco Endoscopy GmbH, Tuebingen, Germany) as a grasping device for all the patients. A 9-mm OTSC was chosen for use in the esophagus and colon, and a 10-mm device was used for the stomach, duodenum and rectum. Clinical success was defined by the results of a computed tomography scan and a blood analysis. Cases considered to be failures were those requiring hemostasis to control GI bleeding. In technical successful cases we included not only complete closing by using OTSCs, but also partial closing where complete closure with OTSCs is almost difficult. In overall clinical successful cases we included only complete closing by using only OTSCs perfectly.

All the OTSCs were placed by 2 experienced endoscopists. The sites closed after ESD included not only the perforation site but also all defect ulcers.

We obtained written, informed consent related to the use of OTSCs from all the patients.

Institution participating in the study

Kagawa University Hospital, Kagawa, Japan, participated in the study.

RESULTS

A total of 23 patients [mean age 77 years (range 64-98 years)] underwent OTSC placement during the study period. Of the 23 patients, 14 were male (60%) and 9 were female (40%). The indications for OTSC placement were GI bleeding (n = 9), perforation (n = 10), fistula (n = 4) and the prevention of post-ESD duodenal artificial ulcer perforation (n = 1). One patient had a perforation caused by a glycerin enema, after which a fistula formed. The perforations that were observed included iatrogenic perforations (n = 8) and hemorrhagic peptic ulcers (n = 2). The iatrogenic perforations included post-ESD artificial ulcer perforations (n = 6), a perforation by a local steroid injection into an ulcer following ESD to prevent gastric stenosis (n = 1), an esophageal perforation by a nasogastric tube (n = 1) (Figure 1) and a rectal perforation by a glycerin enema (n = 1). The fistulae included rectal fistulae (n = 2), a stomach-to-skin fistulae following percutaneous endoscopic gastronomy (PEG) tube removal (n = 1) (Figure 2) and a stomach-to-brachial tube fistula (n = 1) (Figure 3).

The mean maximum size of the lesions was 23.1 mm (range 5 to 50 mm). The lesions were located in the esophagus (n = 1), the stomach (n = 10), the duodenum (n = 5), the sigmoid colon (n = 3) and the rectum (n = 4). The time required for the procedure was measured from the time that the OTSC was applied to the time that it was released in the lesion. The median operation time for the successful cases was 18 min (range 5 to 51 min). The

Table 1 Database of patients who underwent over-the-scope clip device placement

No.	Sex	Age	Location	Primary disease	Maximum lesion size (mm)	Prior treatment history	No. of OTSCs	Operation time (min)	Time from diagnosis (wk)	Technical/overall clinical successful	Additional treatment	Complication	Stay in hospital after OTSC placement (d)	Duration of follow-up (d)
1	M	86	Lower esophagus	Iatrogenic perforation caused by stomach tube	20	None	1	5	< 1	Yes/yes	None	None	6	56
2	M	74	Stomach	Delayed perforation after ESD	40	None	2	24	< 1	Yes/yes	None	None	10	90
3	F	82	Stomach	Perforation after ESD	25	None	2	20	< 1	Yes/yes	None	None	7	8
4	M	80	Stomach	Peptic ulcer with bleeding	15	Hemostatic forceps	1	8	< 1	Yes/yes	None	None	21	30
5	F	71	Stomach	Peptic ulcer with perforation	40	None	2	23	< 1	Yes/yes	None	None	7	58
6	F	88	Stomach	Gastrocutaneous fistula	10	None	1	18	> 4	Yes/yes	None	None	8	84
7	M	98	Stomach	Bleeding due to Mallory-Weiss syndrome	12	None	1	12	< 1	Yes/yes	None	None	6	15
8	M	73	Duodenum	Para-anastomotic ulcer bleeding	15	Clips	2	21	< 1	Yes/yes	None	None	8	18
9	M	80	Duodenal bulb	Peptic ulcer with bleeding	23	Clips and hemostatic forceps	2	30	< 1	Yes/yes	None	None	10	52
10	M	74	Duodenal bulb	Peptic ulcer with perforation	5	None	1	8	< 1	Yes/yes	None	None	13	194
11	F	73	Duodenal bulb	Prevention of post-ESD perforation	25	None	1	10	< 1	Yes/yes	None	None	7	95
12	M	75	3rd portion of duodenum	Delayed perforation after ESD	28	None	2	36	< 1	Yes/yes	None	None	15	210
13	F	85	Rectum	Rectovesical fistula	15	None	2	30	< 1	Yes/yes	None	None	9	28
14	F	88	Rectum	Iatrogenic rectal perforation/ fistula	25	None	1	8	< 1	Yes/yes	None	None	10	30
15	M	73	Stomach	Peptic ulcer with bleeding	20	Clips and HSE	N/A	N/A	1-4	No/no	Hemostatic forceps	N/A	N/A	N/A
16	M	64	Stomach	Peptic ulcer with bleeding	50	Hemostatic forceps	N/A	N/A	1-4	No/no	Hemostatic forceps	N/A	N/A	N/A
17	F	65	Sigmoid colon	Perforation after ESD	35	None	1	7	< 1	Yes/yes	None	None	8	160
18	F	83	Sigmoid colon	Perforation after ESD	40	None	3	16	< 1	Yes/yes	None	None	8	90
19	F	88	Stomach	Perforation caused by a local injection needle	50	None	3	51	1-4	Yes/no	Surgery	N/A	N/A	N/A
20	M	65	Rectum	Postoperative anastomotic ulcer bleeding	5	Hemostatic forceps	1	6	< 1	Yes/yes	None	None	8	14
21	M	65	Rectum	Postoperative anastomotic ulcer bleeding	5	Hemostatic forceps	2	19	< 1	Yes/yes	None	None	7	30
22	M	73	Sigmoid colon	Refractory diverticular bleeding	5	Clips	1	7	< 1	Yes/yes	None	None	5	10
23	M	73	Stomach	Gastrobronchial fistula	28	Bronchial embolization	1	40	> 4	Yes/no	May be given in future	N/A	N/A	N/A

OTSC: Over-the-scope clip; ESD: Endoscopic submucosal dissection; M: Male; F: Female; N/A: Not available; HSE: Hypertonic saline-epinephrine injection therapy.

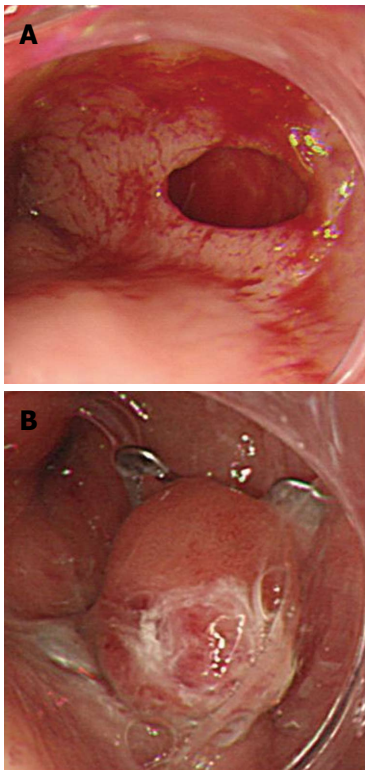

Figure 1 Esophageal perforation caused by a nasogastric tube. A: During the insertion of a nasogastric tube, the tip of the tube perforated the lower esophagus; B: The wound was successfully closed with a single over-the-scope clip, and there was no leakage.

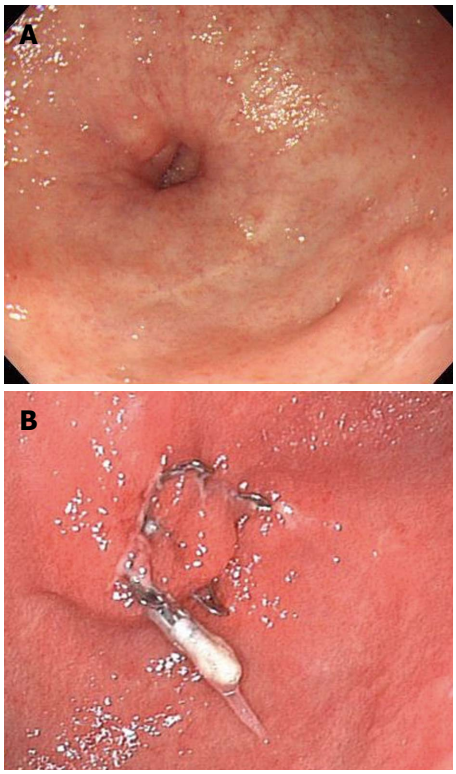

Figure 2 Gastrocutaneous fistula that occurred after percutaneous endoscopic gastronomy removal. A: After the removal of a gastrostomy tube, the patient was able to eat orally, but a gastrocutaneous fistula was diagnosed; B: The wound was slightly hardened, but was successfully closed with a single over-the-scope clip.

numbers of OTSC devices used per session were one (n = 11 patients), two (n = 8 patients) and three (n = 2 patients). The post-OTSC placement mean observation period was 67 d (range 8-210 d).

The technical success rate for OTSC placement was 91.3% (21/23). The OTSC was successfully and safely released in all the cases except for 2, in which we did not release the OTSC because the ulcer was extremely stiff due to being a chronic hemorrhagic gastric ulcer. We could not include the entire thickness of the mucosa of these ulcers within the applicator cap. Ultimately, we stopped the bleeding by reapplying the hemostatic forceps (Coagrasper, FD-410LR, Olympus, Tokyo, Japan). The overall clinical success rate using the OTSC alone was 82.6% (19/23). A large lesion size (greater than 20 mm) and a delayed diagnosis (more than 1 wk) were the major contributing factors in the overall unsuccessful clinical cases. The location of the unsuccessful lesion was in the stomach (Table 2). In 2 patients, we were unable to place the OTSC on the refractory peptic ulcer. In 1 patient, a perforation occurred because of a local steroid injection into the artificial ulcer after ESD to prevent stenosis. We could not place the OTSC correctly because the wound was large and the ulcer was exceptionally stiff. In another patient, who was suffering from a gastrobrachial fistula (Figure 3), we were able to place only one OTSC, but not the additional OTSCs necessary for the complete closure of the fistula.

There was no adjunct therapy used for OTSC placement in the 19 overall clinical success cases. In the two technical failure cases, we used coagulation forceps for additional hemostasis. All the patients were hospitalized for observation after the OTSC placement. The median hospital stay was 9 d (range 6-21 d).

Complications

During the observation period, none of the patients experienced any complications associated with OTSC placement.

DISCUSSION

The development of new NOTES devices has introduced advanced therapeutic techniques that can be used in minimally invasive treatments[1-3], including various full-thickness suturing devices that are applied in clinical practice. One of these devices is the OTSC system, the clinical utility of which has been reported in Europe and the Unites States. The OTSC system shows great potential for use in endoscopic treatments that require speed and simplicity[2,3,17,18]. This system received a pharmaceutical license in Japan in November 2011. Although animal experiments and clinical studies have been performed in Europe and the United States, few clinical cases have been reported in Japan[13,14]. We used the OTSC in NOTES animal experiments prior to its approval for

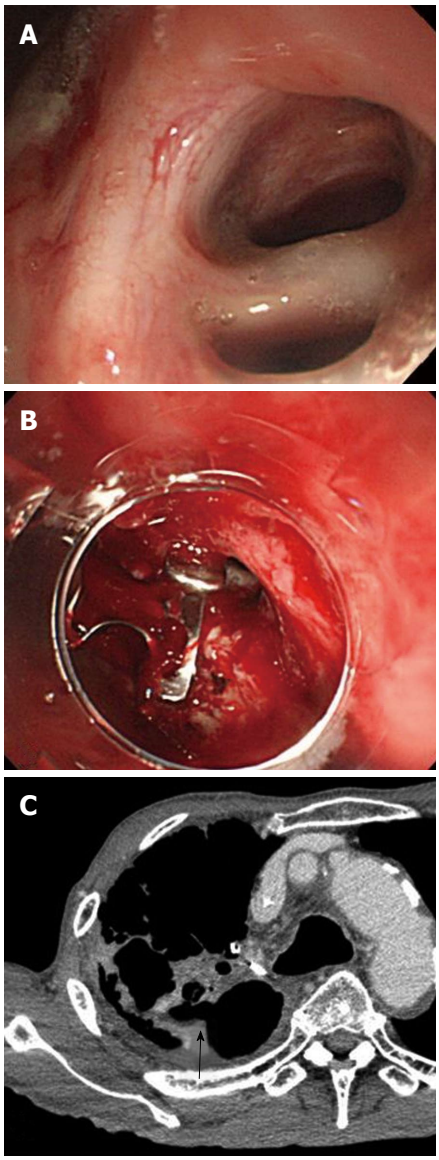

Figure 3 A gastric tube bronchial fistula following a subtotal esophagectomy for esophageal cancer. A: A gastric tube bronchial fistula occurred after a subtotal esophagectomy for esophageal cancer. Bronchial embolization was performed, but it failed to close the fistula; B: The authors attempted to close the fistula using over-the-scope clip (OTSC) but were unsuccessful. Although 1 OTSC was placed, mucosal hardening (resulting from the prolonged duration of the untreated ulcer) prevented the placement of the additional OTSCs required for closure; C: A chest-abdominal computed tomography scan revealed a gastrobronchial fistula (arrow).

humans. Recognizing its potential for use in Japan, we began using the OTSC clinically immediately after the pharmaceutical license was granted. We have employed this device in 23 patients within a short period in our hospital, with an overall success rate of 82%. Animal and clinical studies of the OTSC have demonstrated that gastric, duodenal or colonic perforations up to 15 mm in diameter can be completely closed with a single OTSC. For perforations up to 20 mm in diameter, closure can also be achieved using some OTSCs, which indicates that there is sufficient working space for the unobstructed use of the endoscope during NOTES[17]. There are reports that

Table 2 Relationship between each characters and overall clinical success rate *n* (%)

	Patients	Overall clinical success
Location		
Esophagus	1	1 (100)
Stomach	10	6 (60)
Duodenum	5	5 (100)
Colon	3	3 (100)
Rectum	4	4 (100)
Primary disease		
GI bleeding	9	7 (77)
Chronic fistulae	4	3 (75)
Perforation	11	10 (90)
Maximum lesion size (mm)		
< 20	9	9 (100)
20-30	8	6 (75)
> 30	6	4 (66)
Time from diagnosis (wk)		
< 1	18	18 (100)
1-4	3	0 (0)
> 4	2	1 (50)

full-thickness closures of defects 18-27 mm in diameter can be performed using OTSC; however, it is difficult to completely close defects > 30 mm in diameter[18].

In the present series, OTSC closure was successful for wounds with a maximum diameter of ≤ 30 mm but unsuccessful in 1 case of refractory GI bleeding and 1 case of gastrobronchial fistula (Figure 3). Even in cases with a maximum wound diameter of > 30 mm, we placed the OTSC successfully because the tissue was well extensible and not hardened. In our clinical experience, unsuccessful OTSC closure had a chronic course and OTSC failures were due to chronic fibrotic changes and scarring at the perforation site. Specifically, we used OTSCs in 4 chronic patients and failed to close the perforation site in 3 of them (75%). It appears that wound closure with the OTSC is suitable for wounds with easy extensibility of the surrounding tissues; such lesions have little fibrosis and can be easily grasped by the twin grasper. Considering that successful closure was also achieved in cases with a rectal fistula < 20 mm in diameter or a gastrocutaneous fistula after PEG removal (Figure 2), we believe that OTSC use should be considered when surgery is the only remaining option, provided that the lesion (even if presumably hardened) is ≤ 30 mm in diameter and can be sucked into the cap to lift the mucosa. In addition, the success of the OTSC closures in 4 cases with lesions > 30 mm suggests that this device can be used in acute cases with good extensibility of surrounding mucosa.

ESD was developed for *en bloc* removal of large gastric cancer, which decrease the risk of local recurrence, and specimens can be accurately evaluated by histological examination[19]. However, the procedure is associated with a high incident of complication with bleeding and perforation[20-22]. Now, bleeding has been good controlled with new hemostatic forceps and clips[23]. We also used the OTSC for the complete wound closure of post-ESD perforations in the stomachs of 2 patients who had undergone ESD for early gastric cancer. In both cases,

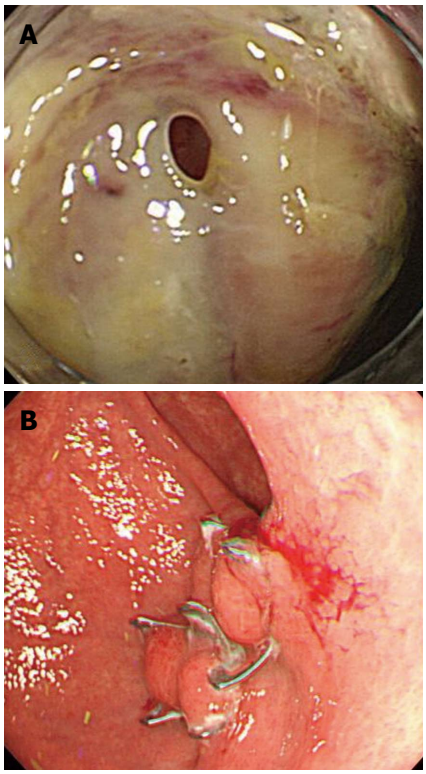

Figure 4 Iatrogenic perforation after endoscopic submucosal dissection for early gastric cancer in the greater curvature of the stomach. A: A post-endoscopic submucosal dissection ulcer was found in the greater curvature of the stomach. An examination by retroflex view revealed that the muscle layer was separated and perforated; B: The wound was successfully closed using an over-the-scope clip (OTSC). An upper endoscopy performed 2 mo after the closure revealed no displacement of the OTSC or complications, such as ulceration or deformation.

the lesions were located in the greater curvature of the stomach (Figure 4). Because it is anatomically thinner than other parts of the stomach, the greater curvature of the stomach is considered to be easy to perforate[24-29]. In addition, because the knife is applied vertically to the mucosa during ESD, it is difficult to perform the procedure while maintaining an appropriate dissection depth into the submucosal layer. The endoscope must also be retroflexed[24]. These limitations have led to several cases in which our attempts to create a partial closure of an ulcer base using conventional clips caused the further extensive separation of the muscle layer and the subsequent need for surgery. In such cases, complete closure using the OTSC is thus preferred for a post-ESD ulcer in the greater curvature of the stomach, even if perforation is suspected.

The use of duodenal ESD is controversial among Japanese endoscopists because the narrow lumen of the duodenum makes it difficult to perform the procedure, and the base of a post-ESD duodenal ulcer is continuously exposed to bile, causing an increased incidence of delayed perforation compared with other ESD sites[30-35]. Nevertheless, duodenal surgery is highly invasive because of the anatomical position. ESD should be preferentially performed instead of surgery if clinically indicated. The

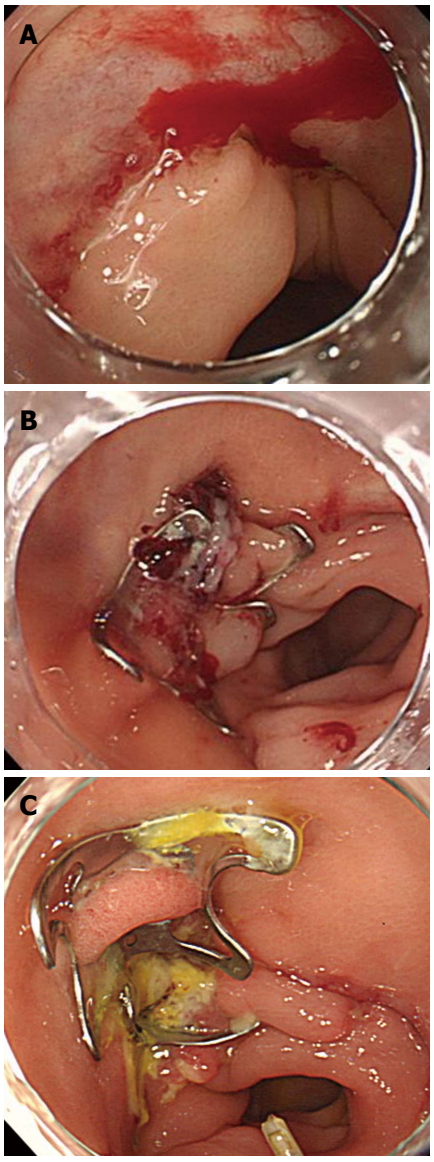

Figure 5 Bleeding from an anastomotic ulcer caused by a sigmoidectomy for sigmoid cancer. A: Bleeding was observed from the anastomotic site after surgery for sigmoid colon cancer; B: The wound was successfully closed using an over-the-scope clip; C: The postoperative course has been uneventful, with no rebleeding.

Japan Gastroenterological Endoscopy Society reported in April 2009 that the complete closure of a post-ESD duodenal ulcer using conventional clips helps prevent delayed perforation. However, conventional clips are too small and do not provide sufficient grip strength to achieve the complete closure of an ulcer base. Therefore, the OTSC, which is larger and provides greater grip strength, is recommended for the complete closure of post-ESD duodenal ulcers[36].

At our hospital, patient 11 (Table 1) experienced a small perforation of a post-ESD ulcer that formed in the duodenal bulb, and the OTSC was used for its closure. In patient 12 (Table 1), the lesion occurred in the descending portion of the duodenum and was exposed to bile, indicating an increased risk of delayed perforation. Thus,

the ulcer was closed using the OTSC, which helped prevent perforation and bleeding.

We experienced 2 cases of OTSC closure for post-ESD ulcers in the colon. In both cases, the lesions were located in the sigmoid colon, with post-ESD perforations requiring complete wound closure. The wound was > 30 mm in diameter in both cases and was successfully closed using the OTSC.

We experienced 9 cases of GI bleeding that were treated with OTSCs. Widely used hemostatic procedures, such as hemostatic clips and local injections, are economically advantageous but sometimes fail to achieve complete primary hemostasis. The use of coagulation hemostasis with hemostatic forceps is also increasing because the reliable coagulation of exposed vessels under direct visualization can minimize the risk of rebleeding. However, the application of coagulation hemostasis to a deep ulcer or a thin wall of the duodenum is associated with a risk of perforation. For patients who do not tolerate surgery well and are in shock due to rebleeding or who do not respond to conventional treatment, the use of the OTSC should be considered. Based on these criteria, we applied OTSCs to the 9 patients with GI bleeding and achieved complete hemostasis in 7 of them. The remaining 2 patients with failed hemostasis using the OTSC system had a personal status ⩾ 3 and could not tolerate open abdominal surgery. One of the patients had a large ulcer (50 mm in diameter) to which hemostasis with hemostatic forceps was applied. However, the patient experienced 2 episodes of shock due to bleeding from other sites of neovascularization. During the third episode of bleeding, ulcer closure with the OTSC was attempted but was unsuccessful due to a hardened ulcer base. Hemostasis with hemostatic forceps was again performed, after which no rebleeding was observed.

Hemostatic treatment of an anastomotic ulcer using the OTSC for was successful in 1 patient with a duodenal lesion and for 2 patients with lesions in the sigmoid colon (Figure 5). The aggressive treatment of bleeding with hemostatic forceps may cause perforations because anastomotic sites are usually thin and fragile. We consider the OTSC to be an effective tool for the treatment of lesions at anastomotic sites.

Regarding safety and complications, none of our patients treated with the OTSC reported any complications. Assessments performed 7 d after closure using the OTSC also revealed no displacements of the OTSC or tissue necrosis at the wound sites. Endoscopic examinations revealed OTSC losses in 2 cases: 1 occurred 1 mo after OTSC placement for duodenal lesions, and the other occurred 2 mo after OTSC placement for colonic lesions. No associated complications were observed in either case. Other possible complications include mucosal damage caused by the teeth of the OTSC protruding out of the hood top during insertion. Therefore, special care should be taken when the OTSC is inserted into physiologically narrow sites, such as the esophageal entrance, pyloric ring or anal ring. There have been no reports of OTSCs causing mucosal deformation or stenosis of the gastrointestine[9]. However, we must consider the possibility that a failure to extract the fibrotic mucosa may result in the tissue being crushed by the twin grasper during the extraction of hardened tissue.

Based on our 23 cases and those reported in the literature, we consider OTSC to be a highly useful device that can be safely utilized in the treatment of GI perforations, fistulae and refractory bleeding. However, OTSC is not suitable for the closure of chronic lesions with hard, severely fibrotic wounds because it is difficult to draw such a lesion into the top of the device. However, we have used the OTSC to close post-ESD perforations with a 100% success rate. Although our sample size was small, we believe that the OTSC is a viable treatment option for post-ESD perforation.

COMMENTS

Background

Recently, over-the-scope clip (OTSC) devices have been used for gastrointestinal (GI) bleeding, fistulae and perforations in the United States and several European countries. OTSC devices became pharmaceutically licensed in Japan in August 2011. The authors have reported their experiences with the OTSC and the outcomes of several cases in Japan. The aims of the present study were to retrospectively review the results of OTSC use in the authors' hospital and to examine the feasibility of using the OTSC to completely close perforations after endoscopic submucosal dissection (ESD) for early GI cancers.

Research frontiers

Historically, the standard treatment for GI perforations has been surgery. Recently, invasive endoscopic treatments, such as ESD and natural orifice transluminal endoscopic surgery (NOTES), have provided alternative approaches to surgery. However, the devices used to treat complications following endoscopic treatments are less convenient and not as safe. OTSCs have been used to treat GI bleeding, fistulae and perforations in several countries. In this study, the authors retrospectively report the results of using the OTSC in their hospital. They also discuss the potential use of the OTSC to completely close perforations after ESD for early GI cancers.

Innovations and breakthroughs

This is the first retrospective study of the OTSC in Japan, and it includes more cases of post-ESD perforation closure compared with other published reports on OTSC. All the post-ESD perforation closure cases in the present study were successes. Thus, the authors consider OTSC to be a possible tool for the treatment of perforations after ESD.

Applications

Based on the present 23 cases and those reported in the literature, the authors assert that the OTSC is useful and safe for the treatment of GI perforations, fistulae and refractory bleeding.

Terminology

ESD is the only nonsurgical, endoscopic method of treating early GI cancers; NOTES, a fusion of flexible endoscopy and operative techniques, is a less invasive form of treatment than surgery.

Peer review

The OTSC is an interesting and novel device that enhances the armamentarium of therapeutic gastroenterologists. This report illustrates the use of this novel device, which facilitates interventions that were previously impossible to perform endoscopically.

REFERENCES

1 **Mori H**, Kobara H, Kobayashi M, Muramatsu A, Nomura T, Hagiike M, Izuishi K, Suzuki Y, Masaki T. Establishment of pure NOTES procedure using a conventional flexible endoscope: review of six cases of gastric gastrointestinal stromal

tumors. *Endoscopy* 2011; **43**: 631-634 [PMID: 21611948 DOI: 10.1055/s-0030-1256227]
2 **von Renteln D**, Vassiliou MC, Rothstein RI. Randomized controlled trial comparing endoscopic clips and over-the-scope clips for closure of natural orifice transluminal endoscopic surgery gastrotomies. *Endoscopy* 2009; **41**: 1056-1061 [PMID: 19899033 DOI: 10.1055/s-0029-1215241]
3 **Voermans RP**, van Berge Henegouwen MI, Bemelman WA, Fockens P. Novel over-the-scope-clip system for gastrotomy closure in natural orifice transluminal endoscopic surgery (NOTES): an ex vivo comparison study. *Endoscopy* 2009; **41**: 1052-1055 [PMID: 19899030 DOI: 10.1055/s-0029-1215231]
4 **Kobara H**, Mori H, Masaki T. Successful en bloc resection of an esophageal hemangioma by endoscopic submucosal dissection. *Endoscopy* 2012; **44** Suppl 2 UCTN: E134-E135 [PMID: 22619035 DOI: 10.1055/s-0030-1256703]
5 **Raju GS**. Endoscopic closure of gastrointestinal leaks. *Am J Gastroenterol* 2009; **104**: 1315-1320 [PMID: 19367272 DOI: 10.1038/ajg.2009.34]
6 **Mori H**, Kobara H, Inoue H, Kobayashi M, Muramatsu A, Nomura T, Gong J, Hagiike M, Izuishi K, Suzuki Y, Masaki T. New technique for safer endoscopic submucosal dissection using the duodenal balloon occlusion method. *J Gastroenterol Hepatol* 2012; **27**: 81-85 [PMID: 21722178 DOI: 10.1111/j.1440-1746.2011.06833.x]
7 **Mori H**, Rafiq K, Kobara H, Fujihara S, Nishiyama N, Kobayashi M, Himoto T, Haba R, Hagiike M, Izuishi K, Okano K, Suzuki Y, Masaki T. Local steroid injection into the artificial ulcer created by endoscopic submucosal dissection for gastric cancer: prevention of gastric deformity. *Endoscopy* 2012; **44**: 641-648 [PMID: 22696191 DOI: 10.1055/s-0032-1309815]
8 **Kobara H**, Mori H, Fujiwara S, Nishiyama N, Kobayashi M, Masaki T. Bloc biopsy by tunneling method using endoscopic submucosal dissection for an upper gastrointestinal submucosal tumor. *Endoscopy* 2012; **44** Suppl 2 UCTN: E197-E198 [PMID: 22622742 DOI: 10.1055/s-0031-1291821]
9 **Traina M**, Curcio G, Tarantino I, Soresi S, Barresi L, Vitulo P, Gridelli B. New endoscopic over-the-scope clip system for closure of a chronic tracheoesophageal fistula. *Endoscopy* 2010; **42** Suppl 2: E54-E55 [PMID: 20157889 DOI: 10.1055/s-0029-1243824]
10 **von Renteln D**, Rudolph HU, Schmidt A, Vassiliou MC, Caca K. Endoscopic closure of duodenal perforations by using an over-the-scope clip: a randomized, controlled porcine study. *Gastrointest Endosc* 2010; **71**: 131-138 [PMID: 19883907 DOI: 10.1016/j.gie.2009.07.006]
11 **Seebach L**, Bauerfeind P, Gubler C. "Sparing the surgeon": clinical experience with over-the-scope clips for gastrointestinal perforation. *Endoscopy* 2010; **42**: 1108-1111 [PMID: 21120779 DOI: 10.1055/s-0030-1255924]
12 **Pohl J**, Borgulya M, Lorenz D, Ell C. Endoscopic closure of postoperative esophageal leaks with a novel over-the-scope clip system. *Endoscopy* 2010; **42**: 757-759 [PMID: 20806160 DOI: 10.1055/s-0030-1255634]
13 **Baron TH**, Song LM, Ross A, Tokar JL, Irani S, Kozarek RA. Use of an over-the-scope clipping device: multicenter retrospective results of the first U.S. experience (with videos). *Gastrointest Endosc* 2012; **76**: 202-208 [PMID: 22726484 DOI: 10.1016/j.gie.2012.03.250]
14 **Albert JG**, Friedrich-Rust M, Woeste G, Strey C, Bechstein WO, Zeuzem S, Sarrazin C. Benefit of a clipping device in use in intestinal bleeding and intestinal leakage. *Gastrointest Endosc* 2011; **74**: 389-397 [PMID: 21612776 DOI: 10.1016/j.gie.2011.03.1128]
15 **Repici A**, Arezzo A, De Caro G, Morino M, Pagano N, Rando G, Romeo F, Del Conte G, Danese S, Malesci A. Clinical experience with a new endoscopic over-the-scope clip system for use in the GI tract. *Dig Liver Dis* 2009; **41**: 406-410 [PMID: 18930700 DOI: 10.1016/j.dld.2008.09.002]
16 **von Renteln D**, Denzer UW, Schachschal G, Anders M, Groth S, Rösch T. Endoscopic closure of GI fistulae by using an over-the-scope clip (with videos). *Gastrointest Endosc* 2010; **72**: 1289-1296 [PMID: 20951989 DOI: 10.1016/j.gie.2010.07.033]
17 **Parodi A**, Repici A, Pedroni A, Blanchi S, Conio M. Endoscopic management of GI perforations with a new over-the-scope clip device (with videos). *Gastrointest Endosc* 2010; **72**: 881-886 [PMID: 20646699 DOI: 10.1016/j.gie.2010.04.006]
18 **von Renteln D**, Schmidt A, Vassiliou MC, Rudolph HU, Caca K. Endoscopic full-thickness resection and defect closure in the colon. *Gastrointest Endosc* 2010; **71**: 1267-1273 [PMID: 20598252]
19 **Ono H**, Kondo H, Gotoda T, Shirao K, Yamaguchi H, Saito D, Hosokawa K, Shimoda T, Yoshida S. Endoscopic mucosal resection for treatment of early gastric cancer. *Gut* 2001; **48**: 225-229 [PMID: 11156645]
20 **Gotoda T**, Yamamoto H, Soetikno RM. Endoscopic submucosal dissection of early gastric cancer. *J Gastroenterol* 2006; **41**: 929-942 [PMID: 17096062]
21 **Imagawa A**, Okada H, Kawahara Y, Takenaka R, Kato J, Kawamoto H, Fujiki S, Takata R, Yoshino T, Shiratori Y. Endoscopic submucosal dissection for early gastric cancer: results and degrees of technical difficulty as well as success. *Endoscopy* 2006; **38**: 987-990 [PMID: 17058162]
22 **Onozato Y**, Ishihara H, Iizuka H, Sohara N, Kakizaki S, Okamura S, Mori M. Endoscopic submucosal dissection for early gastric cancers and large flat adenomas. *Endoscopy* 2006; **38**: 980-986 [PMID: 17058161]
23 **Muraki Y**, Enomoto S, Iguchi M, Fujishiro M, Yahagi N, Ichinose M. Management of bleeding and artificial gastric ulcers associated with endoscopic submucosal dissection. *World J Gastrointest Endosc* 2012; **4**: 1-8 [PMID: 22267977]
24 **Chung IK**, Lee JH, Lee SH, Kim SJ, Cho JY, Cho WY, Hwangbo Y, Keum BR, Park JJ, Chun HJ, Kim HJ, Kim JJ, Ji SR, Seol SY. Therapeutic outcomes in 1000 cases of endoscopic submucosal dissection for early gastric neoplasms: Korean ESD Study Group multicenter study. *Gastrointest Endosc* 2009; **69**: 1228-1235 [PMID: 19249769 DOI: 10.1016/j.gie.2008.09.027]
25 **Yoo JH**, Shin SJ, Lee KM, Choi JM, Wi JO, Kim DH, Lim SG, Hwang JC, Cheong JY, Yoo BM, Lee KJ, Kim JH, Cho SW. Risk factors for perforations associated with endoscopic submucosal dissection in gastric lesions: emphasis on perforation type. *Surg Endosc* 2012; **26**: 2456-2464 [PMID: 22398962 DOI: 10.1007/s00464-012-2211-x]
26 **Toyokawa T**, Inaba T, Omote S, Okamoto A, Miyasaka R, Watanabe K, Izumikawa K, Horii J, Fujita I, Ishikawa S, Morikawa T, Murakami T, Tomoda J. Risk factors for perforation and delayed bleeding associated with endoscopic submucosal dissection for early gastric neoplasms: analysis of 1123 lesions. *J Gastroenterol Hepatol* 2012; **27**: 907-912 [PMID: 22142449 DOI: 10.1111/j.1440-1746.2011.07039.x]
27 **Ohta T**, Ishihara R, Uedo N, Takeuchi Y, Nagai K, Matsui F, Kawada N, Yamashina T, Kanzaki H, Hanafusa M, Yamamoto S, Hanaoka N, Higashino K, Iishi H. Factors predicting perforation during endoscopic submucosal dissection for gastric cancer. *Gastrointest Endosc* 2012; **75**: 1159-1165 [PMID: 22482916 DOI: 10.1016/j.gie.2012.02.015]
28 **Iwabuchi K**. A study of the aging of the stomach: Especially from the view point of mural alteration. *Tokyo Jikeikai Med J* 2008; **123**: 197-210
29 **Jeon SW**, Jung MK, Kim SK, Cho KB, Park KS, Park CK, Kwon JG, Jung JT, Kim EY, Kim TN, Jang BI, Yang CH. Clinical outcomes for perforations during endoscopic submucosal dissection in patients with gastric lesions. *Surg Endosc* 2010; **24**: 911-916 [PMID: 19789921 DOI: 10.1007/s00464-009-0693-y]
30 **Spira IA**, Ghazi A, Wolff WI. Primary adenocarcinoma of the duodenum. *Cancer* 1977; **39**: 1721-1726 [PMID: 322840]

31 **Lim CH**, Park JM, Park CH, Cho YK, Lee IS, Kim SW, Choi MG, Chung IS. Endoscopic submucosal dissection of gastric neoplasia involving the pyloric channel by retroflexion in the duodenum. *Dig Dis Sci* 2012; **57**: 148-154 [PMID: 21842239 DOI: 10.1007/s10620-011-1863-z]
32 **Ono H**, Nonaka S, Uedo N, Kaise M, Oyama T, Doyama H, Kokawa A, Kaneko K, Kodashima S, Tanabe S, Toyonaga T, Hirasawa T, Michida T. Clinical Issues of Duodenal EMR/ESD. *Stomach and Intestine* (Tokyo) 2011; **46**: 1669-1677
33 **Honda T**, Yamamoto H, Osawa H, Yoshizawa M, Nakano H, Sunada K, Hanatsuka K, Sugano K. Endoscopic submucosal dissection for superficial duodenal neoplasms. *Dig Endosc* 2009; **21**: 270-274 [PMID: 19961529 DOI: 10.1111/j.1443-1661.2009.00908.x]
34 **Shinoda M**, Makino A, Wada M, Kabeshima Y, Takahashi T, Kawakubo H, Shito M, Sugiura H, Omori T. Successful endoscopic submucosal dissection for mucosal cancer of the duodenum. *Dig Endosc* 2010; **22**: 49-52 [PMID: 20078665 DOI: 10.1111/j.1443-1661.2009.00917.x]
35 **Kirschniak A**, Subotova N, Zieker D, Königsrainer A, Kratt T. The Over-The-Scope Clip (OTSC) for the treatment of gastrointestinal bleeding, perforations, and fistulas. *Surg Endosc* 2011; **25**: 2901-2905 [PMID: 21424197 DOI: 10.1007/s00464-011-1640-2]
36 **Mori H**, Shintaro F, Kobara H, Nishiyama N, Rafiq K, Kobayashi M, Nakatsu T, Miichi N, Suzuki Y, Masaki T. Successful closing of duodenal ulcer after endoscopic submucosal dissection with over-the-scope clip to prevent delayed perforation. *Dig Endosc* 2012 Aug 7; Epub ahead of print [PMID: 23368742 DOI: 10.1111/j.1443-1661.2012.01363.x]

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ORIGINAL ARTICLE

Lipoic acid suppresses portal endotoxemia-induced steatohepatitis and pancreatic inflammation in rats

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Abstract

AIM: To examine the effect of α-lipoic acid (LA) on mild portal endotoxemia-induced steatohepatitis and associated pancreatic abnormalities in fructose-fed rats.

METHODS: Rats were randomly assigned into two groups with a regular or 60% fructose-enriched diet for 8 wk. After fructose feeding for 4 wk, rats were further divided into four subgroups: with intraportal saline (F_{PV}), with intraportal saline plus administration of LA (F_{PV+LA}), with lipopolysaccharide (LPS) infusion (F_{PLPS}), and with LPS infusion plus administration of LA ($F_{PLPS+LA}$). Rats were treated with LPS using intraportal infusion while LA was administered orally. Metabolite levels, superoxide levels, inflammatory markers, malondialdehyde content, glutathione content and toll-like receptor 4 (*TLR4*) gene expression were all measured using standard biochemical techniques. Pancreatic insulin secretion was evaluated by a hyperglycemic clamp technique. Histology of liver and pancreas tissues were evaluated using hematoxylin and eosin staining and immunohistochemistry.

RESULTS: Fructose-induced elevation in plasma C-reactive protein, amylase, superoxide, white blood cell count as well as in hepatic and pancreatic contents of malondialdehyde, tumor necrosis factor alpha and interleukin-6 were increased in animals treated with LPS and reversed with LA administration. The augmented hepatic gene expression of *TLR4* in fructose-fed rats was further increased in those with intraportal LPS infusion, which was partially reversed by LA administration. Pathological examination showed inflammatory changes and leukocyte infiltration in hepatic and pancreatic islets of animals treated with LPS but were rarely observed in those with LA treatment. In addition to affects on the liver, impaired pancreatic insulin secretion seen in fructose-fed rats was deteriorated in with LPS treatment and partially reversed with LA administration.

CONCLUSION: These data suggest LA could significantly suppress mild portal-endotoxemia but not fructose-induced liver and pancreatic abnormalities in a rodent model for metabolic syndrome.

Key words: Lipoic acid; Oxidative stress; Steatohepatitis; Portal endotoxemia; Insulin secretion; Fructose

Core tip: α-lipoic acid (LA), a potent antioxidant and also an inducer of endogenous antioxidants has been

reported to protect the liver and pancreas from injury. Our observations suggest that LA could significantly suppress inflammatory change of steatosis induced by low-dose intraportal lipopolysaccharide infusion and associated deterioration of insulin secretion in this metabolic syndrome rodent model. In addition, our data suggest that hepatic toll-like receptor 4 signaling might not only play a significant role in chronic fructosefeeding-induced hepatic steatosis but also in its subacute inflammatory change induced by mild portal endotoxemia and associated extrahepatic disorders.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is currently the most common liver disease in the world[1]. NAFLD is mainly associated with obesity, diabetes, hyperlipidemia and insulin resistance, which are the main characters of metabolic syndrome[1,2]. The development of liver inflammation in NAFLD, defined as non-alcoholic steatohepatitis (NASH), isone of the crucial steps in causing liver-related morbidity and mortality[3]. Moreover, a pathological link between portal endotoxemia and NASH and also alcoholic liver disease has been speculated in several animal and human studies[4-8]. For instance, genetically obese fatty/fatty rats and *ob*/*ob* mice showed increased sensitivity to endotoxin hepatotoxicity, quickly developing steatohepatitis after exposure to low doses of lipopolysaccharide (LPS)[5]. These studies implicate that portal endotoxemia might significantly contribute to the pathogenesis of chronic hepatic inflammation, especially in NAFLD.

The release of liver-derived acute-phase proteins and inflammatory cytokines under chronic liver injury significantly contributes to the extrahepatic effect of inflamed liver and the pathogenesis of metabolic syndrome[9,10]. We have shown that mild portal endotoxemia induced by low-dose intraportal LPS infusion could significantly cause chronic hepatic and pancreatic inflammation, and impair pancreatic insulin secretion in rats[11]. Furthermore, low-dose intraportal LPS infusion could also accelerate the process of NAFLD to NASH in fructose-fed rats, an animal model of metabolic syndrome with NAFLD[4]. However, the possible underlying mechanisms behind the detrimental effects of mild portal endotoxemia on liver and pancreas remain unclear.

Chronic stress such as portal endotoxemia has been documented to activate hepatic Kupffer cells and cause the release of reactive oxygen species, potentially inducing inflammatory changes in the liver and impairing pancreatic functions[5,12]. α-lipoic acid (LA) is a potent antioxidant and an inducer of endogenous antioxidants[13,14]. It also has a protective effect on hepatic and pancreatic injury[15-18]. In this study, we sought to test the potential therapeutic effect of LA on mild portal endotoxemia and fructose-induced inflammatory changes of fatty liver and impaired pancreatic insulin secretion in rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats aged five to six weeks were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). The rats were housed in an animal center certified by Association of Assessment and Accreditation of Laboratory Animal Care. All animals were handled according to the guidelines and manual of the institutional animal care and use committee of this institute. Rats weighing 250-300 g were randomly assigned into six groups: (1) two control groups: on regular chow diets for 4 wk and then separated into those combined with or without intraportal vehicle (saline) infusion (C_{PV} *vs* C) for another 4 wk; (2) four experimental groups: on high-fructose enriched diet (60% of the calories from fructose, TD89247, Teklad Primer Labs, Madison, WI) for 4 wk and thereafter (F_{PV}), those combined with intraportal vehicle or low-dose LPS infusion (F_{PLPS}), cotreated with vehicle or LA (α-LA in saline, pH 7.8, Sigma Co. Germany, 60 mg/kg per day, oral gavage)for additional 4 wk (F_{PV+LA} and $F_{PLPS+LA}$ respectively)[19]. In the time-course study, blood samples were taken by tail bleeding method after overnight fasting. At the end of week 8, one set of rats from the above grouping (n = 6 per group) was used for an *in vivo* hyperglycemic clamp study. The second set (n = 6 per group) was euthanized by overdose pentobarbital injection (100 mg/kg, intraperitoneal) immediately after overnight fasting and blood sampling by cardiac puncture was carried out for the measurement of plasma C-reactive protein (CRP), superoxide, white blood cell (WBC) count and endotoxin levels. Tissue samples were dissected for further analysis. The liver and pancreas dissected from the third set of rats (n = 6 per group) were fixed by perfusion with 4% paraformadehyde. The tissues were embedded in paraffin for further staining. Notably, the metabolic and hemodynamic parameters and histopathological examination of group C_{PV} were similar to those without intraportal vehicle infusion (data not shown). The group C_{PV} was used as the only control group on regular chow diet in the following result section.

Intraportal infusion

A laparotomy was performed under anesthesia (sodium pentobarbital 25 mg/kg, intraperitoneal injection) in rats with intraportal infusion. A catheter (PE-5 tubing, 0.008 inch inner diameter × 0.020 inch outer diameter, SCI micro medical grade polyethylene; Scientific Commodities Inc., Lake Havasu City, AZ, United States) was inserted

into the distal end of a colic vein and the tip of the catheter was placed about 3 mm distal to the point at which the catheterized vein enters portal vein. After insertion, the catheters were filled with LPS-saline solution or saline alone and were connected to osmotic mini-pumps (model 2004, Alzet corporation, Cupertino, CA, United States) filled with LPS, 0.42 ng/kg per minute or saline as shown in our previous study[20]. Three days before the surgery, a safe dosage of LA was orally administered once per day for 4 wk[21,22].

Hyperglycemic clamp

In one set of rats, vascular catheters were placed in the left femoral artery and right femoral vein under anesthesia and their proximal ends were placed in subcutaneous pockets under scapular area at the end of week 8. The hyperglycemic clamp experiment was performed after recovery for 3 to 4 d, as described elsewhere[23,24]. The insulin secretions of the first and second phases were indicated by the incremental plasma insulin values during time 0-10 and 10-240 min in the glucose clamp period, respectively.

Plasma metabolic parameters

The WBC count was determined by using a Bright-Line-hemocytometer (Hausser Scientific, Horsham, PA, United States). Whole blood glucose levels were measured by the glucose oxidase method. Plasma and tissue triglyceride levels were determined by using appropriate enzymatic colorimetric method (Randox Laboratories, Antrium, United Kingdom). Plasma insulin levels were determined by commercial rat enzyme-linked immuno sorbent assay (ELISA) kits (Mercodia AB, Uppsala, Sweden). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), albumin and amylase levels were measured by Randox reagent kits (Randox Laboratories Antrim, United Kingdom). CRP levels were measured by a commercial rat ELISA kit (Alpha Diagnostic, San Antonio, TX, United States). Arterial plasma endotoxin level was assayed by using the limulus amoebocyte lysate test (Kinetic-QLC; Whittaker Bioproducts, Walkersville, MD, United States).

Plasma superoxide levels

A lucigenin-dependent chemiluminescence assay (Sigma Chemical Co. St. Louis, MO, United States) was used to quantify plasma superoxide levels with the MLA-GOLDS chemiluminescence analyzing system (Tohoku Electronic Industrial Co., Sendai, Japan) as described previously[4]. The total amount of chemiluminescence was calculated by integrating the area under the curve and subtracting it from the background level during the 10 min counting period.

Tissue tumor necrosis factor alpha and interleukin-6 contents

Tissues were prepared as described previously[25]. The supernatant was subjected to tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) assays using commercial rat ELISA kits (R and D Systems, Minneapolis, MN, United States).

Tissue malondialdehyde content

Lipid oxidation of the liver and pancreas was detected by a commercial malondialdehyde (MDA) assay kit (Cayman Chemical Co., Ann Arbor, MI, United States). In brief, MDA, the breakdown product of oxidative degradation of lipids, reacted with thiobarbituric acid (TBAR) to form MDA-TBAR, and its levels were expressed as nmol/g protein.

Tissue glutathione content

The total glutathione in the liver was measured by using commercial glutathione assay kit (Cayman Chemical Co., Ann Arbor, MI, United States). In brief, the liver was homogenized in RIPA buffer (0.5 mol/L Tris-HCl, pH 7.4, 1.5 mol/L NaCl, 2.5% deoxycholic acid, 10% NP-40, 10 mmol/L EDTA) by using a Polytron homogenizer to obtain tissue lysates and centrifuged at 10000 *g* for 10 min. Subsequently, the supernatant was collected and added HPO_3 (v/v = 1/1) to deproteinize. The glutathione (GSH) content of deproteinized supernatant of liver (50 L) was measured by the reductive rate of 5,5'-dithio-*bis*-2-nitrobenzoic acid to 5-thio-2-nitrobenzoic acid according to the instructions with absorption at 405 nm. The GSH level was expressed as μmol/g protein.

Toll-like receptor 4 gene expression in the liver and pancreas

RNA was extracted from the liver and pancreas of experimental rats using Trizol (Ambion, Austin, TX, United States) at the end of the study. TaqMan gene expression assay kits for toll-like receptor 4 (TLR4) (Rn00569848_m1) and the TATA box binding protein (Rn01455648_m1) were used in conjunction with a universal master mix in a 7500 real-time polymerase chain reaction system (Applied Biosystems, Foster City, CA, United States). Gene expression was normalized to the TATA box binding protein expression level measured in each sample and expressed as fold increases or decreases from control values for each gene of interest.

Hematoxylin and eosin stain

The third set of rats (n = 5-6 per group) was grouping as the hyperglycemic clamp experiment. The perfused liver and pancreas were then fixed in 5% zinc-formalin solution and embedded in paraffin. These tissue slices were then prepared for staining with hematoxylin and eosin (HE) (liver, pancreas) to evaluate pathological changes.

Immune cell immunohistochemistry and scoring

Tissue samples were fixed in formalin, and cut into 4 μm sections. Immunohistochemistry was performed on an automated stainer (Autostainer; Dako, Glostrup, Denmark). Then, sections were incubated with anti-CD68 antibody (ED1 mouse anti-rat CD68, Serotec, 1:100, MorphoSys UK Ltd., Oxford, United Kingdom), followed by incubation with goat anti-mouse secondary antibody and

Table 1 Metabolic and hemodynamic parameters during pump infusion period in rats

	Pump infusion (wk)	CPV	FPV	FPV + LA	FPLPS	FPLPS + LA
Body weight (g)	4	349 ± 19	383 ± 8	351 ± 29	358 ± 6	371 ± 6
	8	451 ± 7	474 ± 8	445 ± 29	436 ± 11	423 ± 7
Glucose (mmol/L)	4	5.9 ± 0.3	5.9 ± 0.1	6.62 ± 1.2	6.08 ± 0.1	6.1 ± 0.1
	8	5.9 ± 0.2	6.4 ± 0.2	7.8 ± 0.7	6.76 ± 0.2	6.43 ± 0.1
Insulin (ng/mL)	4	0.5 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
	8	0.6 ± 0.1	2.5 ± 0.6[a]	2.2 ± 0.3[a]	1.9 ± 0.5[a]	2.4 ± 0.4[a]
TG (mg/dL)	4	58 ± 4	180 ± 14[a]	185 ± 13[a]	185 ± 24[a]	163 ± 16[a]
	8	52 ± 4	324 ± 38[a]	286 ± 22[a]	308 ± 24[a]	249 ± 44[a]
AST (U/L)	4	65 ± 5	76 ± 1	82 ± 6	70 ± 2	77 ± 2
	8	58 ± 4	72 ± 2	73 ± 6	68 ± 2	70 ± 2
ALT (U/L)	4	53 ± 2	57 ± 1	56 ± 0	52 ± 1	51 ± 2
	8	56 ± 2	49 ± 1	44 ± 6	46 ± 1	51 ± 1
Albumin (mmol/L)	4	40 ± 1	35 ± 1	37 ± 1	35 ± 0	36 ± 0
	8	41 ± 1	35 ± 0	34 ± 1	35 ± 0	35 ± 0
CRP (μg/mL)	8	397 ± 10	504 ± 10[a]	555 ± 39	583 ± 13[c]	529 ± 22[e]
Amylase (U/L)	8	1278 ± 138	1750 ± 23[a]	1753 ± 58	2149 ± 31[c]	1744 ± 16[e]
Superoxide (count/min)	8	1337 ± 68	3169 ± 24[a]	3860 ± 52	6069 ± 60[c]	3234 ± 33[e]
WBC count (/mm^3)	8	14168 ± 586	20290 ± 390[a]	22622 ± 630	24357 ± 1008[c]	21442 ± 767[e]
Plasma endotoxin (EU/mL)	8	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02

Rats were on regular (C) or high-fructose enriched diet (F) for 4 wk and then further divided into those with intraportal infusion of saline or lipopolysaccharides (LPS), combined with vehicle or α lipoic acid (LA) for additional 4 wk (CPV, FPV, FPV + LA, FPLPS, FPLPS + LA). Values are expressed as mean ± SE, n = 6 per group. [a]P < 0.05 *vs* CPV; [c]P < 0.05 *vs* FPV; [e]P < 0.05 *vs* FPLPS for each corresponding time point. TG: Triglycerides; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; CRP: C-reactive protein; WBC: White blood cell.

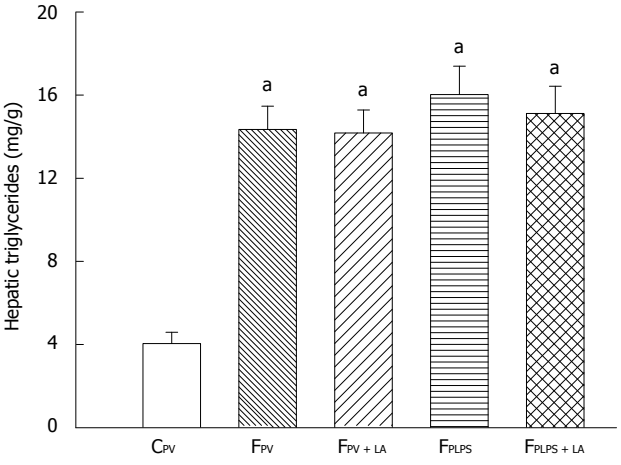

Figure 1 Effects of α-lipoic acid on hepatic triglyceride contents. Hepatic triglyceride levels were measured from the blood of each group of fructose-fed rats and the control group. Values are expressed as mean ± SE, n = 6 per group, [a]P < 0.05 *vs* CPV. C: Regular diet; F: High-fructose enriched diet; LA: Lipoic acid; LPS: Lipopolysaccharides.

visualized with HRP substrate (REAL EnVision Detection System; Dako).

A total of 100 ± 25 islets per experimental group were blindly scored for CD68-positive cells around the periphery and/or within islets from five or six different animals by two investigators. Islet area was measured as the area of islet capsule in pancreatic section with HE stain and computed using AxioVision LE 4.8.2.0 software.

Statistical analysis

Statistical analysis was performed according to the repeated measurements of one-way analysis of variance followed by Bonferroni test. A probability of P < 0.05 was taken to indicate a significant difference between means. Values are expressed as mean ± SE.

RESULTS

Metabolic and hemodynamic parameters

We first measured the effect of fructose feeding and LPS treatment on rats with and without LPS infusion. After high-fructose feeding for 4 wk, fasting plasma insulin levels in fructose-fed groups were significantly increased as compared with controls, but not different among fructose-fed groups. Plasma CRP, amylase, superoxide and white blood cells were also significantly increased in fructose-fed rats and further increased after intraportal LPS infusion for 4 wk. The above LPS-induced responses were suppressed in those co-treated with LA to levels similar to those in fructose-fed untreated controls. On the other hand, there were no significant differences in fasting plasma glucose, AST, ALT, albumin and endotoxin concentrations among experimental groups (Table 1).

Triglyceride contents, oxidative parameters and inflammatory parameters in hepatic tissue

To measure the liver response to our conditions, we measured triglyceride levels, oxidative parameters, and inflammatory parameters. Fructose feeding resulted in elevated triglyceride levels in all groups when compared to the control groups. Administration of LA had no effect on these levels (Figure 1). As shown in Figure 2A, MDA levels in the liver were significantly increased in fructose-fed rats as compared with controls and further elevated in animals treated with LPS. However, the increase of

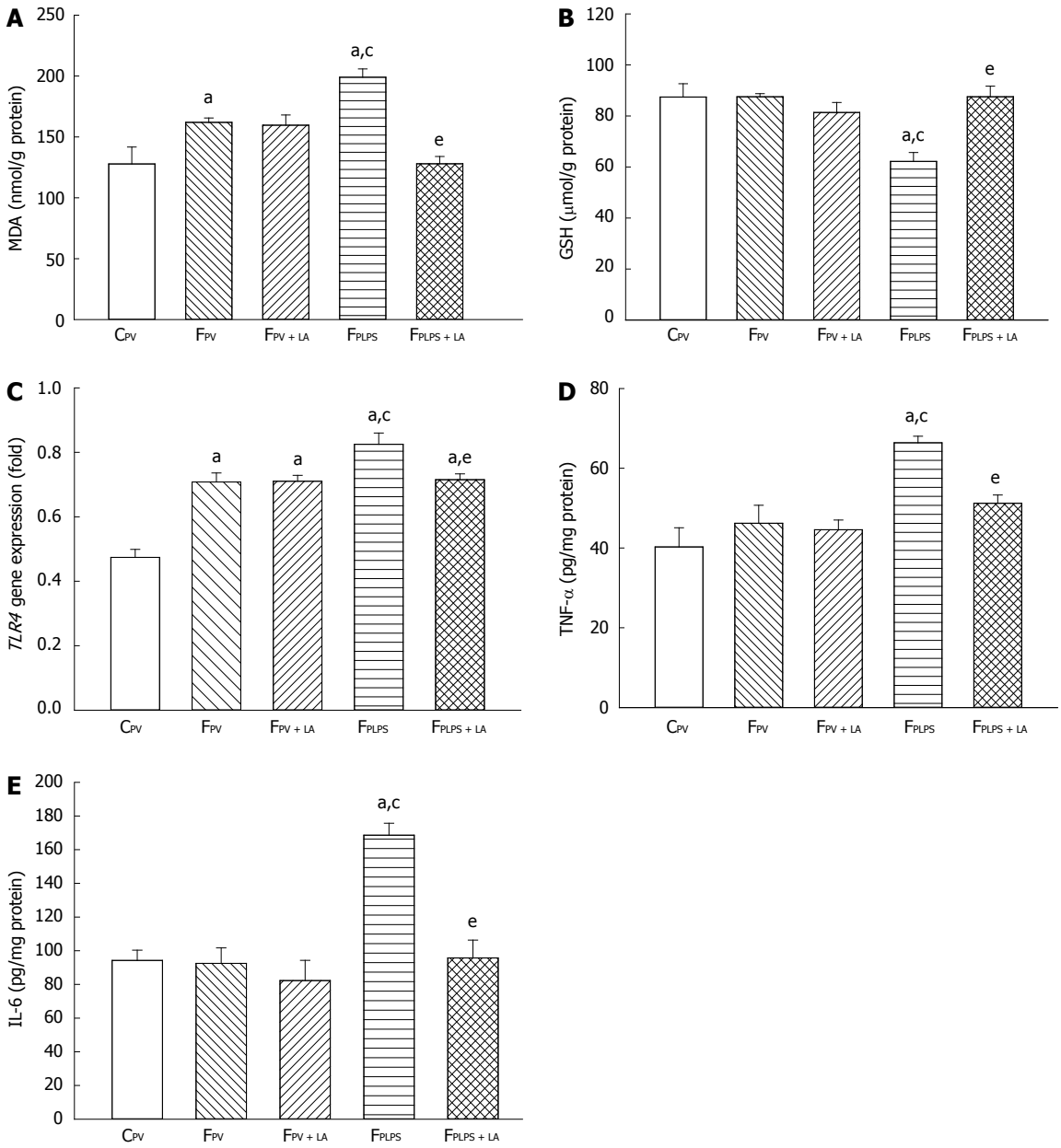

Figure 2 Effect of α-lipoic acid on portal endotoxemia-induced changes in hepatic oxidative and inflammatory markers. The following parameters were measured from liver of each group of fructose-fed rats and the control group: (A) Malondialdehyde (MDA) content; (B) Glutathione (GSH) content; (C) Toll-like receptor 4 (*TLR4*) gene expression; (D) Tumor necrosis factor alpha (TNF-α) protein; and (E) Interleukin-6 (IL-6) protein. Values are expressed as mean ± SE, $n = 6$ per group. $^{a}P < 0.05$ *vs* CPV; $^{c}P < 0.05$ *vs* FPV; $^{e}P < 0.05$ *vs* FPLPS. C: Regular diet; F: High-fructose enriched diet; LA: Lipoic acid; LPS: Lipopolysaccharides.

hepatic MDA contents in fructose-fed rats following intraportal LPS infusion was significantly reversed in those with LA administration. Liver GSH levels were significantly decreased following LPS infusion, but were significantly reversed in those treated with LA (Figure 2B). Additionally, the enhanced hepatic *TLR4* gene expression by chronic high-fructose feeding was further increased in those with intraportal LPS infusion. LA administration suppressed the augmentation of *TLR4* gene expression induced only by mild portal endotoxemia and not by high-fructose feeding (Figure 2C). The significant increase of TNF-α and IL-6 protein levels in the liver of LPS-infused rats was reflective of the hepatic inflammatory response. These levels were significantly suppressed with LA treatment (Figure 2D and E). LPS infusion had no effect on triglyceride levels, but did have an effect on MDA, GSH, TLR4, TNF-α and IL-6. LA administration significantly reversed these effects.

Histopathological changes in the liver

Following analysis of the liver response to our treatments, we sought to examine the liver for histopathological changes. Not surprisingly, steatosis was noted in fructose-fed groups but was not different between groups. On the other hand, the infiltration of monocytes in liver was markedly exhibited in LPS-treated animals (Figure 3D), a phenotype not seen in any other treatment group (Figure 3A-C), and alleviated by the administration of LA

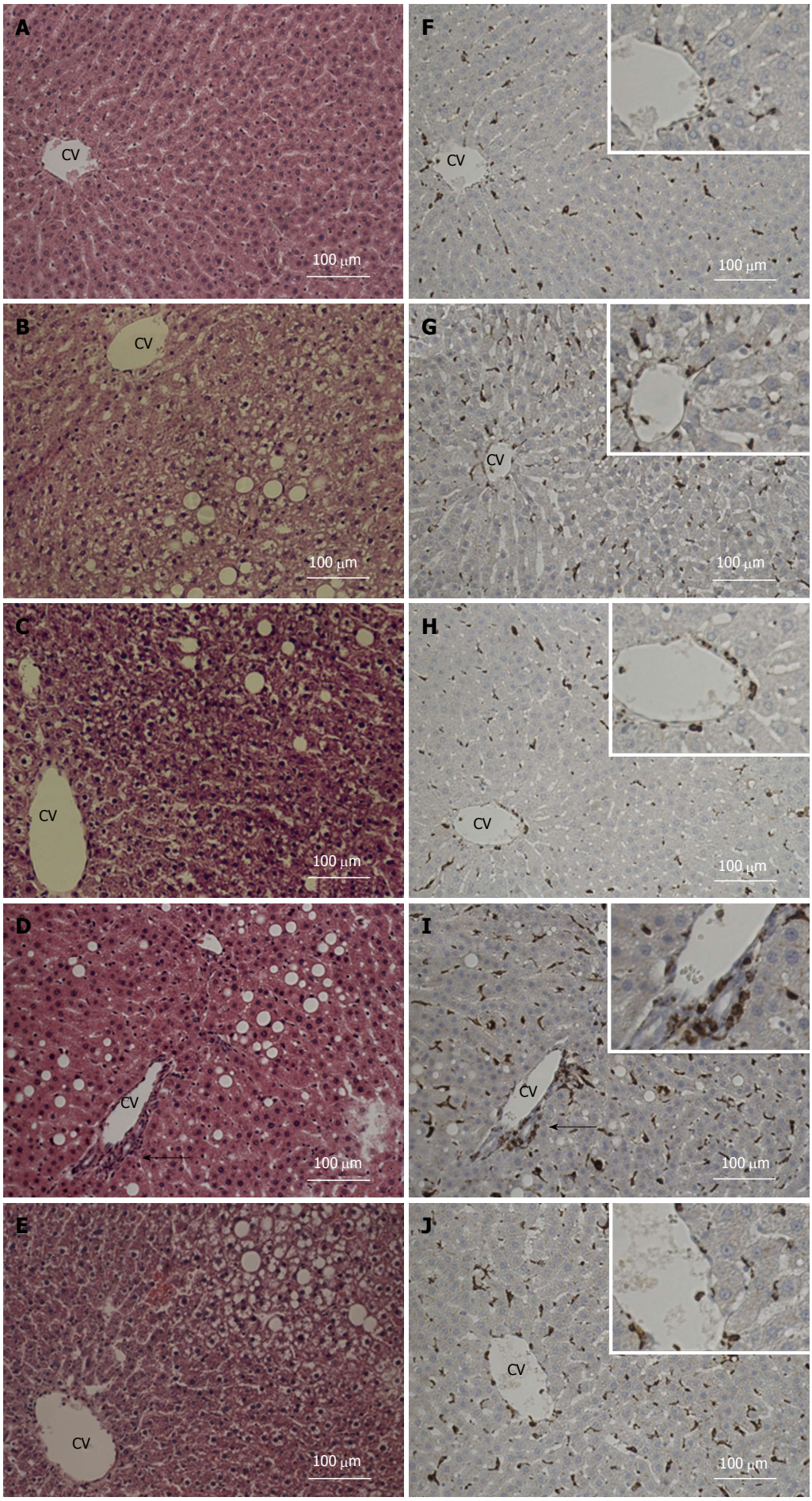

Figure 3 Histopathological examination of liver in rats with regular or high-fructose feeding. Lipid accumulation in: (A) C_{PV} rats; (B) F_{PV} rats; (C)F_{PV+LA}; (D) F_{PLPS}; and (E) $F_{PLPS+LA}$ rats. CD-68 positive cell infiltration in: (F) C_{PV} rats; (G) F_{PV} rats; (H) F_{PV+LA}; (I) F_{PLPS}; and (J) $F_{PLPS+LA}$ rats. Slides were stained with hematoxylin and eosin (A-E) and immunostained with an anti-CD68 antibody (F-J). Arrows indicate CD68 positive cells. C: Regular diet; F: High-fructose enriched diet; LA: Lipoic acid; LPS: Lipopolysaccharides; CV: Central vein.

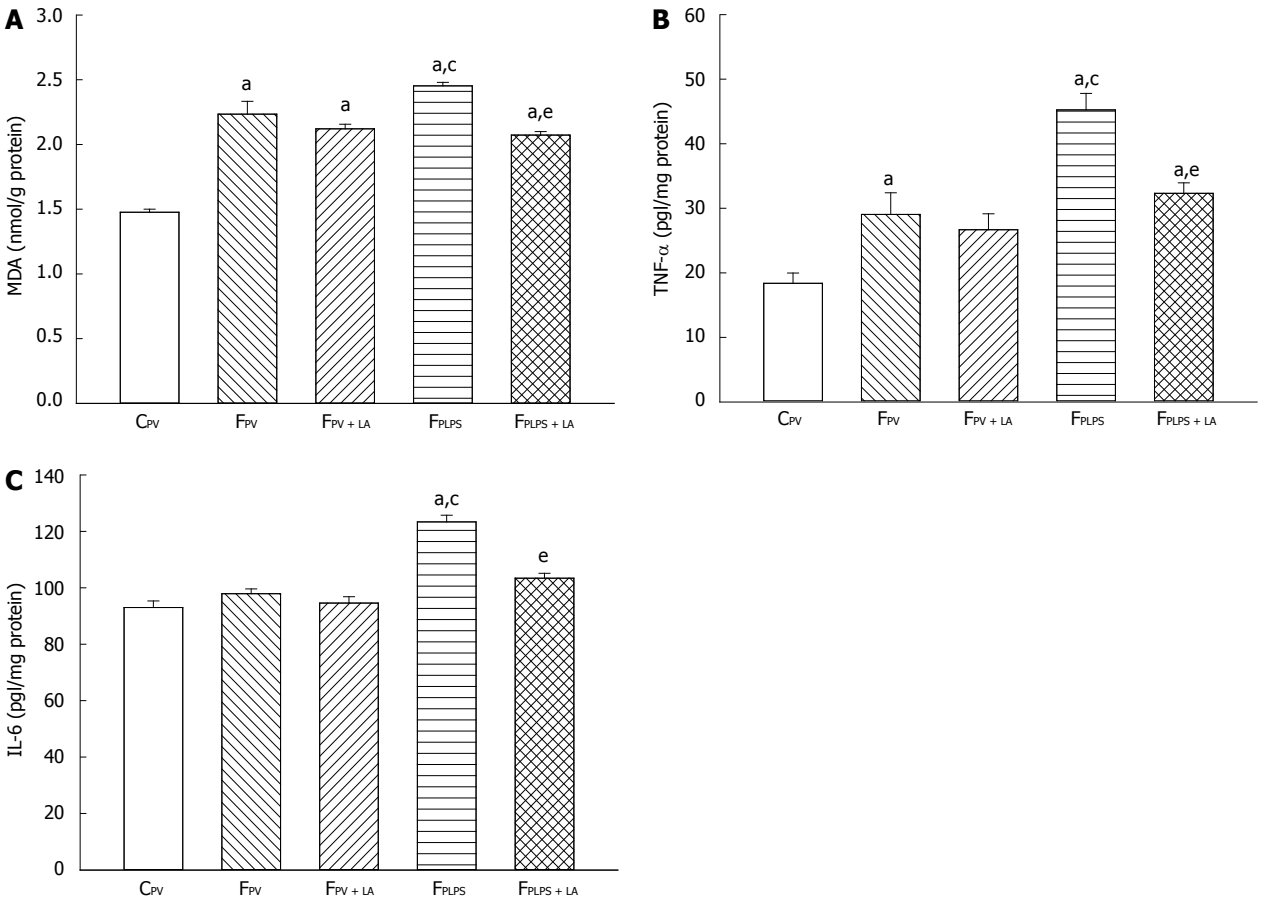

Figure 4 Effect of α-lipoic acid on portal endotoxemia-induced changes in the pancreas. The pancreata from experimental animals were harvested and tested for: (A) Malondialdehyde (MDA) content; (B) Tumor necrosis factor alpha (TNF-α) protein level; and (C) Interleukin-6 (IL-6) protein level. Values are expressed as mean ± SE, $n = 6$ per group. $^{a}P < 0.05$ *vs* CPV; $^{c}P < 0.05$ *vs* FPV; $^{e}P < 0.05$ *vs* FPLPS. C: Regular diet; F: High-fructose enriched diet; LA: Lipoic acid; LPS: Lipopolysaccharides.

(Figure 3E). Accordingly, immunohistochemical staining showed that the observed CD68-positive cell infiltration was observed in areas around the central vein in LPS infused animals (Figure 3I), but significantly attenuated in those with LA administration (Figure 3J). Therefore, LPS treatment resulted in marked histopathological changes in the liver of fructose-fed animals, a phenotype that was reversed by the administration of LA.

Content of MDA and inflammatory markers in the pancreas

To measure the pancreas response to our conditions, we tested for MDA, TNF-α and IL-6 levels. Fructose feeding increased all three parameters, while fructose feeding with infusion of LPS significantly increased each (Figure 4A-C). Administration of LA resulted in each parameter returning back to original fructose-fed levels. *TLR4* gene expression was not detected in pancreas in experimental groups. Therefore, immunological markers including TNF-α and IL-6, but not TLR4, are increased upon fructose feeding and LPS infusion, a phenotype that can be reversed with administration of LA.

Histopathologic changes in the pancreatic islets

We then examined the histopathological response to our treatments in the pancreas. Following LPS treatment, pancreatic islets exhibited damage that was attenuated with LA administration (Figure 5, top panels). Consistently, the frequency and CD68-positive cell infiltration in fructose-fed animals was only slightly increased as compared to controls. However, they were markedly increased in following LPS infusion and attenuated with LA administration (Figure 5, bottom panels). Quantitation of this data shows the frequency of CD68-positive islets and numbers of CD68-positive cells/islet in fructose-fed rats were further elevated following LPS infusion and partially suppressed in those with LA administration.

Glucose-stimulated insulin secretion

Hyperglycemic clamp is the gold-standard method to evaluate glucose-stimulated insulin secretion *in vivo*[26]. Plasma glucose levels were not different during the basal period and maintained similar hyperglycemia during the clamp periods among experimental groups (Figure 6A). Increases in plasma insulin levels from baseline in fructose-fed animals were significantly lower than those in controls and further decreased in those infused with LPS under similar hyperglycemic conditions (Figure 6B). The diminished glucose-stimulated insulin secretion shown in fructose-fed controls was not significantly changed with

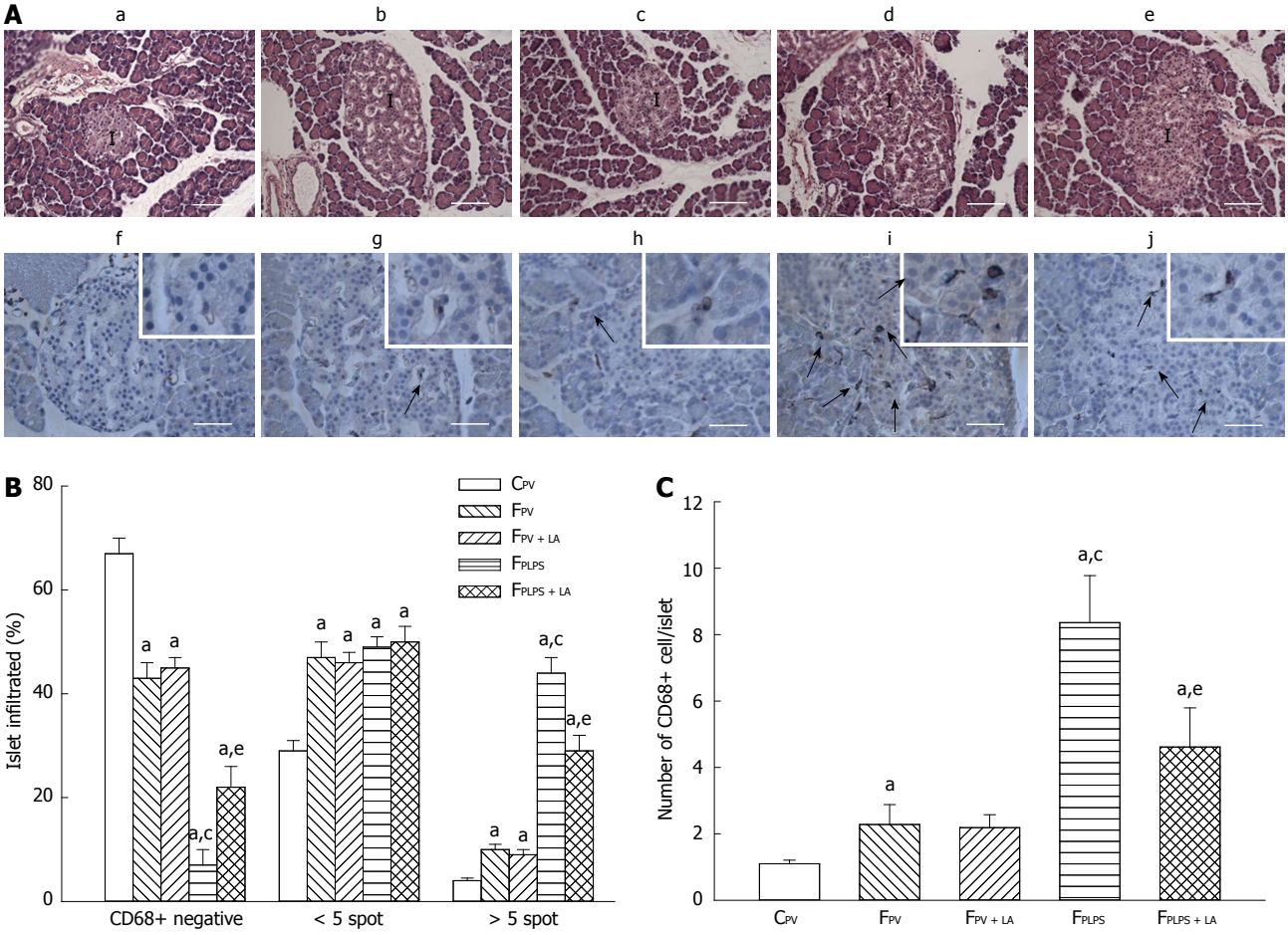

Figure 5 Histopathologicalexamination of pancreatic islets. A: Tissue samples from each subset of animals were analyzed by hematoxylin and eosin (HE) staining (a-C_{PV}, b-F_{PV}, c-F_{PV+LA}, d-F_{PLPS}, e-$F_{PLPS+LA}$) and immunostaining with an antibody against CD68 (f-C_{PV}, g-F_{PV}, h-F_{PV+LA}, i-F_{PLPS}, j-$F_{PLPS+LA}$); This data was then quantitated as (B) percentage of islets infiltrated and (C) number of CD68+ cells per islet. Arrows indicated CD68-positive cells. I: Islet. A total of 100 ± 25 islets per experimental group were blindly scored for CD68-positive cells around the periphery and/or within islets from 5 to 6 different animals. Islet area was measured by the area of islet capsule in pancreatic section with HE stain and computed using AxioVision LE 4.8.2.0 software. Values are expressed as mean ± SE, n = 6 per group. Line bar: 50 μm. $^{a}P < 0.05$ *vs* C_{PV}; $^{c}P < 0.05$ *vs* F_{PV}; $^{e}P < 0.05$ *vs* F_{PLPS}. C: Regular diet; F: High-fructose enriched diet; LA: Lipoic acid; LPS: Lipopolysaccharides.

LA administration. However, intraportal LPS infusion significantly impaired the glucose-stimulated insulin secretion shown in fructose-fed controls, which was significantly reversed in those with LA treatment (Figure 6B). In addition, the first-phase insulin secretion (0-10 min post glucose treatment) and the second-phase insulin secretion (10-240 min post glucose treatment) were significantly lower in fructose-fed animals when compared with controls. This was further exacerbated following intraportal LPS infusion, a phenotype that was partially reversed when treated with LA (Figure 6C).

DISCUSSION

Nonalcoholic steatohepatitis is not only highly correlated with the development of metabolic syndrome and type 2 diabetes mellitus, but is also a crucial factor in progression to cirrhosis and hepatocellular carcinoma[3]. In addition, mild portal endotoxemia has been speculated as a crucial risk factor to induce hepatic inflammation in the state of steatosis and impaired pancreatic β cell function[4]. This study explored the potential therapeutic role of the potent antioxidant α-LA in liver disease and associated pancreatic abnormalities. Using the animal model for metabolic steathohepatitis, fructose-fed rats, we found administration of LA reversed mild portal endotoxemia-induced inflammation. Further, portal endotoxemia also decreased glucose-stimulated insulin secretion, a phenotype that was reversed following LA treatment.

LA has been reported to scavenge free radicals, chelate metals and restore intracellular GSH, all factors associated with increasing age[13]. In addition, it is used as a therapeutic agent in several liver-related diseases such alcohol-induced liver damage[27] and fatty liver disease[16] through multiple mechanisms. Our observations further establish LA as a therapeutic for liver disease. Specifically, our animal model shows LA improves endotoxin-induced hepatic disorders in addition to its established role as a treatment for chronic high-fructose feeding. These antioxidant and anti-inflammatory characteristics may be in the ability of LA to restore tissue GSH-dependent antioxidant defenses during portal endotoxin attack.

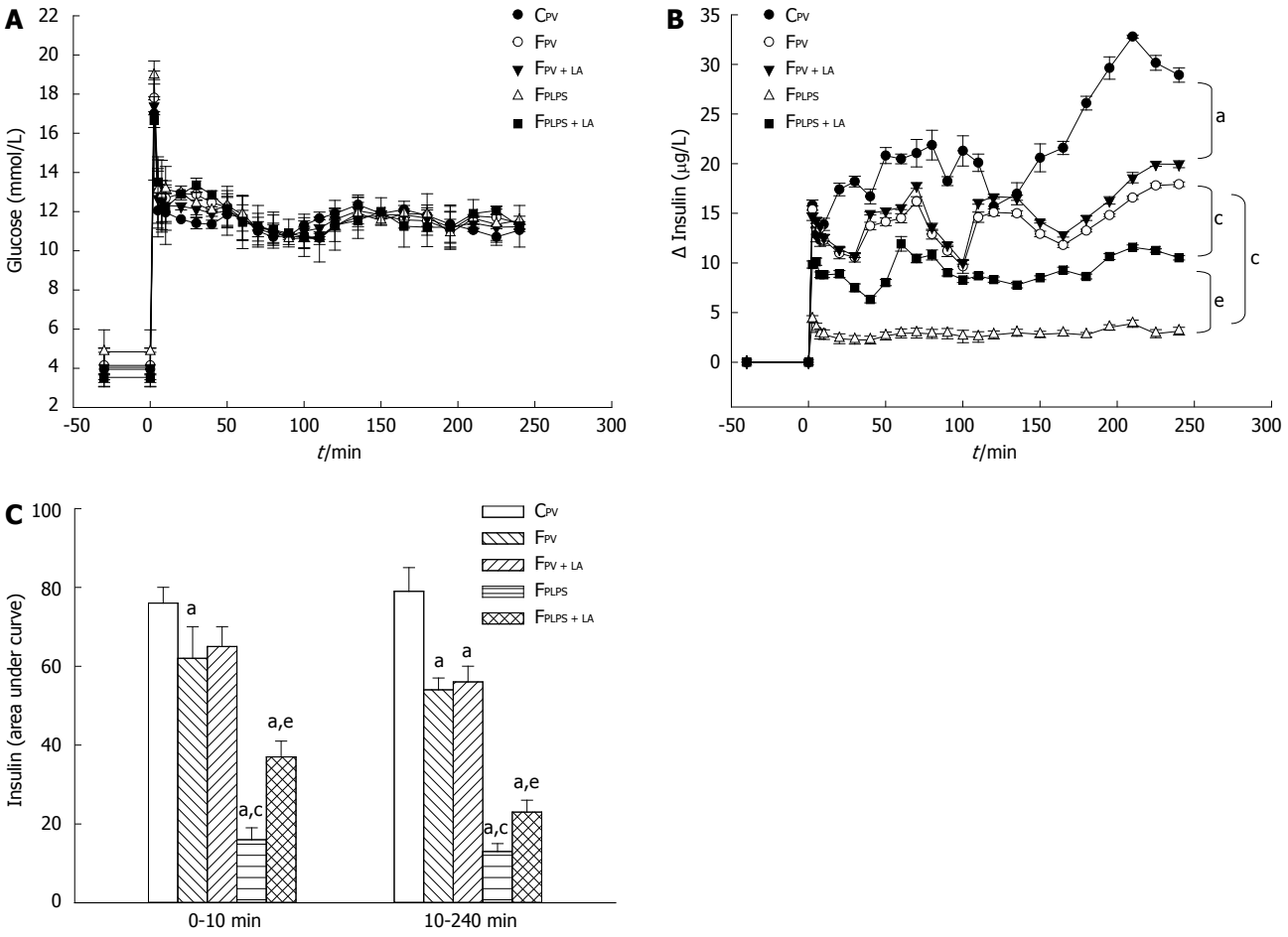

Figure 6 Effect of α-lipoic acid on plasma glucose and insulin levels. A hyperglycemic clamp technique was used to evaluate glucose-stimulated insulin secretion. A: Plasma glucose levels during the clamp period; B: Change in insulin levels during clamp period; C: The insulin level averages during the first phase (0-10 min) and the second phase (10-240 min) of the clamp period. Values are expressed as mean ± SE, $n = 6$ per group. $^{a}P < 0.05$ *vs* C_{PV}; $^{c}P < 0.05$ *vs* F_{PV}; $^{e}P < 0.05$ *vs* F_{PLPS}. C: Regular diet; F: High-fructose enriched diet; LA: Lipoic acid; LPS: Lipopolysaccharides.

Furthermore, these data show LA administration significantly suppressed the augmented *TLR4* gene expression induced by mild portal endotoxemia but not chronic fructose feeding. Hepatic TLR4 is activated by gut-derived LPS and attributed to the pathogenesis of liver inflammation[28]. In addition, TLR4 signaling is involved in the development of fructose-induced steatosis in mice[29] and also required for liver steatosis, inflammation and a fibrogenic response after chronic alcohol treatment in TLR4 transgenic mice[30]. In addition to LPS, other potential agents including saturated fatty acids (*i.e.*, palmitate) and alarmins (*i.e.*, HMGB1) have also been shown to stimulate the inflammatory response in a TLR4-dependent manner[31,32]. Hepatic TLR4 signaling occurs not only in Kupffer cells but also in hepatic non-immune cell populations including hepatocytes, biliary epithelial cells, endothelial cells and hepatic stellate cells[33]. In brief, these observations implicate TLR4-mediated inflammatory signaling in hepatic cell populations are crucially involved in the development of NAFLD, affected individually by diet components and portal endotoxemia.

Our observations showed that LA not only significantly improved the inflammatory changes in fructose-induced NASH but also diminished the detrimental inflammatory response of the pancreas. Consistent with our results, LA has a protective effect on cholecystokinin-octapeptide induced acute pancreatitis in rats[15]. LA was reported to have a dose-related cytoprotective effect on hydrogen peroxide-induced oxidative stress on pancreatic beta cells. On the other hand, LA could also directly suppress insulin secretion in pancreatic beta cells at high concentrations by inducing AMP-activated protein kinase activation[34]. Taken together, data from these studies combined with our observations implicate a beneficial effect of LA on impaired pancreatic insulin secretion. This effect might be attributed to its anti-oxidative and anti-inflammatory actions on mild portal endotoxemia-induced liver inflammation and subsequent pancreatic damage, but not its direct effect on pancreatic beta cell secretion.

Nevertheless, the selected dose of intraportal LPS infusion was based on evaluating its effects on the mortality rate and systemic endotoxin levels during 4-wk infusion period in our previous study[11]. Consistently, this low-dose LPS infusion caused subacute hepatic inflammation that could be almost completely be cleared by Kupffer cells once it passed through the liver, so that the arterial

plasma endotoxin levels were not different among experimental groups.

The present study showed that administration of LA could protect liver from LPS-induced oxidative stress and inflammation while reversing the subsequent impairment of the pancreas in rats with fructose-associated fatty liver disease. However, LA had no effect on oxidative stress induced by high-fructose feeding. Clinical implications of this observation suggest both the suppression of portal endotoxemia-induced oxidative stress and dietary interventions are crucial for improving symptoms of NASH.

Although both oxidative stress induced by low-dose intraportal LPS infusion and high-fructose feeding have been demonstrated to contribute to the development of steatohepitis in fructose-fed rats, the possible involvement of TLR4 signaling in individual hepatic cell populations remain elusive in the present study.

In conclusion, the present study demonstrates that LA can significantly reduce intraportal LPS-induced liver inflammation and associated deterioration of insulin secretion function in this metabolic syndrome rodent model. In addition, it is also implicated that hepatic TLR4 signaling might not only play a significant role in chronic-fructose-feeding induced hepatic steatosis but also in its subacute inflammatory change induced by mild portal endotoxemia and associated extrahepatic disorders in this model.

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COMMENTS

Background

α-lipoic acid (LA), a potent antioxidant and also an inducer of endogenous antioxidants has been reported to protect the liver and pancreas from injury.

Research frontiers

Chronic stress such as portal endotoxemia has been documented to activate hepatic Kupffer cells and cause the release of reactive oxygen species, potentially inducing inflammatory changes in the liver and impairing pancreatic functions. The potential therapeutic effect of LA on mild portal endotoxemia and fructose-induced inflammatory changes of fatty liver and impaired pancreatic insulin secretion in rats remain controversial.

Innovations and breakthroughs

LA has been documented to have a protective effect on hepatopancreatic damage. However, the effect of LA on lipopolysaccharide-induced non-alcoholic steatohepatitis (NASH) remains unclear. The present result in this manuscript has a significant contribution to clarify this issue.

Applications

The present study implicates that LA might suppress inflammatory change of steatosis and associated deterioration of insulin secretion in the patients with metabolic syndrome.

Terminology

Alpha LA is a potent antioxidant and an inducer of endogenous antioxidants. It also has a protective effect on hepatic and pancreatic injury.

Peer review

The authors found that LA quenches inflammation and oxidative stress, leading to attenuation of NASH. So far a few reports describe the effect of LA on lipopolysaccharide-induced NASH. The manuscript represents a major effort to fill the gap.

REFERENCES

1 **Marchesini G**, Marzocchi R. Metabolic syndrome and NASH. *Clin Liver Dis* 2007; **11**: 105-117 [PMID: 17544974 DOI: 10.1016/j.cld.2007.02.013]
2 **Moscatiello S**, Di Luzio R, Sasdelli AS, Marchesini G. Managing the combination of nonalcoholic fatty liver disease and metabolic syndrome. *Expert Opin Pharmacother* 2011; **12**: 2657-2672 [PMID: 22043839 DOI: 10.1517/14656566.2011.629188]
3 **Gambino R**, Musso G, Cassader M. Redox balance in the pathogenesis of nonalcoholic fatty liver disease: mechanisms and therapeutic opportunities. *Antioxid Redox Signal* 2011; **15**: 1325-1365 [PMID: 20969475 DOI: 10.1089/ars.2009.3058]
4 **Hsieh PS**. Inflammatory change of fatty liver induced by intraportal low-dose lipopolysaccharide infusion deteriorates pancreatic insulin secretion in fructose-induced insulin-resistant rats. *Liver Int* 2008; **28**: 1167-1175 [PMID: 18397237 DOI: 10.1111/j.1478-3231.2008.01714.x]
5 **Yang SQ**, Lin HZ, Lane MD, Clemens M, Diehl AM. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. *Proc Natl Acad Sci USA* 1997; **94**: 2557-2562 [PMID: 9122234]
6 **Packer L**, Kraemer K, Rimbach G. Molecular aspects of lipoic acid in the prevention of diabetes complications. *Nutrition* 2001; **17**: 888-895 [PMID: 11684397 DOI: 10.1016/S0899-9007(01)00658-X]
7 **Brun P**, Castagliuolo I, Di Leo V, Buda A, Pinzani M, Palù G, Martines D. Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G518-G525 [PMID: 17023554 DOI: 10.1152/ajpgi.00024.2006]
8 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102]
9 **Müller S**, Martin S, Koenig W, Hanifi-Moghaddam P, Rathmann W, Haastert B, Giani G, Illig T, Thorand B, Kolb H. Impaired glucose tolerance is associated with increased serum concentrations of interleukin 6 and co-regulated acute-phase proteins but not TNF-alpha or its receptors. *Diabetologia* 2002; **45**: 805-812 [PMID: 12107724 DOI: 10.1007/s00125-002-0829-2]
10 **Pickup JC**, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia* 1997; **40**: 1286-1292 [PMID: 9389420]
11 **Hsieh PS**, Chan JY, Shyu JF, Chen YT, Loh CH. Mild portal endotoxaemia induces subacute hepatic inflammation and pancreatic beta-cell dysfunction in rats. *Eur J Clin Invest* 2008; **38**: 640-648 [PMID: 18837740 DOI: 10.1111/j.1365-2362.2008.01991.x]
12 **Reid AE**. Nonalcoholic steatohepatitis. *Gastroenterology* 2001; **121**: 710-723 [PMID: 11522755 DOI: 10.1053/gast.2001.27126]
13 **Shay KP**, Moreau RF, Smith EJ, Smith AR, Hagen TM. Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential. *Biochim Biophys Acta* 2009; **1790**: 1149-1160 [PMID: 19664690 DOI: 10.1016/j.bbagen.2009.07.026]
14 **Bast A**, Haenen GR. Lipoic acid: a multifunctional antioxidant. *Biofactors* 2003; **17**: 207-213 [PMID: 12897442]
15 **Park SJ**, Seo SW, Choi OS, Park CS. Alpha-lipoic acid protects against cholecystokinin-induced acute pancreatitis in rats. *World J Gastroenterol* 2005; **11**: 4883-4885 [PMID: 16097064]
16 **Park KG**, Min AK, Koh EH, Kim HS, Kim MO, Park HS, Kim YD, Yoon TS, Jang BK, Hwang JS, Kim JB, Choi HS, Park JY, Lee IK, Lee KU. Alpha-lipoic acid decreases hepatic lipogenesis through adenosine monophosphate-activated protein kinase (AMPK)-dependent and AMPK-independent pathways. *Hepatology* 2008; **48**: 1477-1486 [PMID: 18972440 DOI: 10.1002/hep.22496]

17 **Abdin AA**, El-Hamid MA, El-Seoud SH, Balaha MF. Effect of pentoxifylline and/or alpha lipoic acid on experimentally induced acute pancreatitis. *Eur J Pharmacol* 2010; **643**: 289-296 [PMID: 20599924 DOI: 10.1016/j.ejphar.2010.06.020]

18 **Bustamante J**, Lodge JK, Marcocci L, Tritschler HJ, Packer L, Rihn BH. Alpha-lipoic acid in liver metabolism and disease. *Free Radic Biol Med* 1998; **24**: 1023-1039 [PMID: 9607614 DOI: 10.1016/S0891-5849(97)00371-7]

19 **Cani PD**, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmée E, Cousin B, Sulpice T, Chamontin B, Ferrières J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007; **56**: 1761-1772 [PMID: 17456850 DOI: 10.2337/db06-1491]

20 **Curat CA**, Miranville A, Sengenès C, Diehl M, Tonus C, Busse R, Bouloumié A. From blood monocytes to adipose tissue-resident macrophages: induction of diapedesis by human mature adipocytes. *Diabetes* 2004; **53**: 1285-1292 [PMID: 15111498 DOI: 10.2337/diabetes.53.5.1285]

21 **Cremer DR**, Rabeler R, Roberts A, Lynch B. Long-term safety of alpha-lipoic acid (ALA) consumption: A 2-year study. *Regul Toxicol Pharmacol* 2006; **46**: 193-201 [PMID: 16899332 DOI: 10.1016/j.yrtph.2006.06.003]

22 **Cremer DR**, Rabeler R, Roberts A, Lynch B. Safety evaluation of alpha-lipoic acid (ALA). *Regul Toxicol Pharmacol* 2006; **46**: 29-41 [PMID: 16904799 DOI: 10.1016/j.yrtph.2006.06.004]

23 **Wagoner B**, Hausman DB, Harris RB. Direct and indirect effects of leptin on preadipocyte proliferation and differentiation. *Am J Physiol Regul Integr Comp Physiol* 2006; **290**: R1557-R1564 [PMID: 16424081 DOI: 10.1152/ajpregu.00860.2005]

24 **Marques BG**, Hausman DB, Latimer AM, Kras KM, Grossman BM, Martin RJ. Insulin-like growth factor I mediates high-fat diet-induced adipogenesis in Osborne-Mendel rats. *Am J Physiol Regul Integr Comp Physiol* 2000; **278**: R654-R662 [PMID: 10712285]

25 **Ghanim H**, Garg R, Aljada A, Mohanty P, Kumbkarni Y, Assian E, Hamouda W, Dandona P. Suppression of nuclear factor-kappaB and stimulation of inhibitor kappaB by troglitazone: evidence for an anti-inflammatory effect and a potential antiatherosclerotic effect in the obese. *J Clin Endocrinol Metab* 2001; **86**: 1306-1312 [PMID: 11238525 DOI: 10.1210/jc.86.3.1306]

26 **Belfort R**, Harrison SA, Brown K, Darland C, Finch J, Hardies J, Balas B, Gastaldelli A, Tio F, Pulcini J, Berria R, Ma JZ, Dwivedi S, Havranek R, Fincke C, DeFronzo R, Bannayan GA, Schenker S, Cusi K. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006; **355**: 2297-2307 [PMID: 17135584 DOI: 10.1056/NEJMoa060326]

27 **Packer L**, Witt EH, Tritschler HJ. alpha-Lipoic acid as a biological antioxidant. *Free Radic Biol Med* 1995; **19**: 227-250 [PMID: 7649494 DOI: 10.1016/0891-5849(95)00017-R]

28 **Lin Y**, Yu LX, Yan HX, Yang W, Tang L, Zhang HL, Liu Q, Zou SS, He YQ, Wang C, Wu MC, Wang HY. Gut-derived lipopolysaccharide promotes T-cell-mediated hepatitis in mice through Toll-like receptor 4. *Cancer Prev Res* (Phila) 2012; **5**: 1090-1102 [PMID: 22617167 DOI: 10.1158/1940-6207.CAPR-11-0364]

29 **Spruss A**, Kanuri G, Wagnerberger S, Haub S, Bischoff SC, Bergheim I. Toll-like receptor 4 is involved in the development of fructose-induced hepatic steatosis in mice. *Hepatology* 2009; **50**: 1094-1104 [PMID: 19637282 DOI: 10.1002/hep.23122]

30 **Inokuchi S**, Tsukamoto H, Park E, Liu ZX, Brenner DA, Seki E. Toll-like receptor 4 mediates alcohol-induced steatohepatitis through bone marrow-derived and endogenous liver cells in mice. *Alcohol Clin Exp Res* 2011; **35**: 1509-1518 [PMID: 21463341 DOI: 10.1111/j.1530-0277.2011.01487.x]

31 **Shi H**, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest* 2006; **116**: 3015-3025 [PMID: 17053832 DOI: 10.1172/JCI28898]

32 **Klune JR**, Dhupar R, Cardinal J, Billiar TR, Tsung A. HMGB1: endogenous danger signaling. *Mol Med* 2008; **14**: 476-484 [PMID: 18431461 DOI: 10.2119/2008-00034.Klune]

33 **Seki E**, Brenner DA. Toll-like receptors and adaptor molecules in liver disease: update. *Hepatology* 2008; **48**: 322-335 [PMID: 18506843 DOI: 10.1002/hep.22306]

34 **Lee BW**, Kwon SJ, Chae HY, Kang JG, Kim CS, Lee SJ, Yoo HJ, Kim JH, Park KS, Ihm SH. Dose-related cytoprotective effect of alpha-lipoic acid on hydrogen peroxide-induced oxidative stress to pancreatic beta cells. *Free Radic Res* 2009; **43**: 68-77 [PMID: 19358002 DOI: 10.1080/10715760802590400]

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ORIGINAL ARTICLE

Oncogene *GAEC1* regulates *CAPN10* expression which predicts survival in esophageal squamous cell carcinoma

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Abstract

AIM: To identify the downstream regulated genes of *GAEC1* oncogene in esophageal squamous cell carcinoma and their clinicopathological significance.

METHODS: The anti-proliferative effect of knocking down the expression of *GAEC1* oncogene was studied by using the RNA interference (RNAi) approach through transfecting the *GAEC1*-overexpressed esophageal carcinoma cell line KYSE150 with the pSilencer vector cloned with a *GAEC1*-targeted sequence, followed by MTS cell proliferation assay and cell cycle analysis using flow cytometry. RNA was then extracted from the parental, pSilencer-*GAEC1*-targeted sequence transfected and pSilencer negative control vector transfected KYSE150 cells for further analysis of different patterns in gene expression. Genes differentially expressed with suppressed *GAEC1* expression were then determined using Human Genome U133 Plus 2.0 cDNA microarray analysis by comparing with the parental cells and normalized with the pSilencer negative control vector transfected cells. The most prominently regulated genes were then studied by immunohistochemical staining using tissue microarrays to determine their clinicopathological correlations in esophageal squamous cell carcinoma by statistical analyses.

RESULTS: The RNAi approach of knocking down gene expression showed the effective suppression of *GAEC1* expression in esophageal squamous cell carcinoma cell line KYSE150 that resulted in the inhibition of cell proliferation and increase of apoptotic population. cDNA microarray analysis for identifying differentially expressed genes detected the greatest levels of downregulation of calpain 10 (*CAPN10*) and upregulation of trinucleotide repeat containing 6C (*TNRC6C*) transcripts when *GAEC1* expression was suppressed. At the tissue level, the high level expression of calpain 10 protein was significantly associated with longer patient survival (month) of esophageal squamous cell carcinoma compared to the patients with low level of calpain 10 expression (37.73 ± 16.33 *vs* 12.62 ± 12.44, $P = 0.032$). No significant correction was observed among the TNRC6C protein expression level and the clinocopathologcial features of esophageal squamous cell carcinoma.

CONCLUSION: *GAEC1* regulates the expression of

CAPN10 and *TNRC6C* downstream. Calpain 10 expression is a potential prognostic marker in patients with esophageal squamous cell carcinoma.

Key words: Esophageal squamous cell carcinoma; Oncogene; RNA interference; Calpain 10; Tissue microarray

Chan D, Tsoi MYT, Liu CD, Chan SH, Law SYK, Chan KW, Chan YP, Gopalan V, Lam AKY, Tang JCO. Oncogene *GAEC1* regulates *CAPN10* expression which predicts survival in esophageal squamous cell carcinoma. *World J Gastroenterol* 2013; 19(18): 2772-2780 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i18/2772.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i18.2772

INTRODUCTION

Esophageal squamous cell carcinoma (ESCC) has a multifactorial etiology which involves environmental and/or genetic factors[1,2]. The incidence of ESCC also shows marked variation in its geographic distribution and occurs at relatively high frequency in Asian regions including China[3]. Current modalities of therapy for this disease offer relatively poor survival and cure rates[4], thus more investigations at the molecular level are essential for a better understanding the molecular pathogenesis of this disease and for making further improvements in diagnosis and treatment of ESCC.

Gene amplification and overexpression have been suggested as the major genomic aberrations involved in the pathogenesis of ESCC[5,6]. We previously employed the method of comparative DNA fingerprinting using inter-simple sequence repeat polymerase chain reaction (ISSR-PCR) and revealed that amplifications or deletions of chromosomal sequences are common events in both the preneoplastic lesions and carcinomas[7]. An analysis of the frequency of amplification or loss of individual ISSR-PCR profile bands led to the identification of a novel expressed sequence tag database entry of a cDNA clone from a chromosome 7 placental cDNA library[7,8]. Moreover, the ISSR-PCR fragment also showed 98% homology to a Homo sapiens chromosome 7 P1-derived artificial chromosome clone (approximately 125 kb) which has been mapped to chromosome band 7q22[9]. The amplification of chromosomal segment 7q22 has been implicated in many types of cancer. Reported examples include ESCC[10], breast carcinoma[11], pancreatic carcinoma[12], renal-cell carcinomas[13] and T-cell leukemia[14]. Thus, further investigation on the newly identified ESCC-related genomic and expressed sequences mapped to chromosomal region 7q22 can be a fruitful approach for identifying new candidate genes crucial to the disease. We subsequently identified and characterized the role of a novel oncogene *GAEC1* which is located at 7q22 region, encodes a nuclear protein and shows a high frequency of gene amplification and overexpression in ESCC cell lines and primary tumors[15], as well as in colorectal adenocarcinoma[16]. Overexpression of *GAEC1* in 3T3 mouse fibroblasts caused increased cell proliferation, foci formation and colony formation in soft agar, comparable to *H-ras* overexpression. Further, injection of *GAEC1*-transfected 3T3 cells into athymic nude mice formed undifferentiated sarcoma *in vivo*, providing the first evidence about the oncogenic nature of *GAEC1*[15]. An increased *GAEC1* DNA copy number was also reported in 79% of colorectal adenocarcinomas and the copy numbers were significantly different among colorectal adenocarcinomas, adenomas, and non-neoplastic colorectal tissues[16].

In this report, *GAEC1* was further characterized by identifying the downstream partners using cDNA microarray analysis on *GAEC1*-suppressed human esophageal carcinoma cell line KYSE150 which shows *GAEC1* overexpression. The prominently downstream-regulated genes were then studied by immunohistochemistry on a tissue microarray (TMA) of ESCC to determine their clinicopathological significance.

MATERIALS AND METHODS

ESCC specimens and cell lines

One hundred and thirty-two paired non-tumor and tumor fresh tissue samples were collected after esophagectomy with patients' consent at the Department of Surgery, Queen Mary Hospital, Hong Kong from 2001 to 2006. They were collected consecutively from esophagectomy specimens performed on patients who had received no prior treatment directed to the primary ESCC. The histopathological features were reported by specialist pathologists of the Department of Pathology, Queen Mary Hospital, Hong Kong. The clinicopathological parameters of the patients were collected prospectively and they included age, gender, tumor-node-metastasis pathological stages and histological grades. The actuarial survival rate of the patients was calculated from the date of surgical resection of the ESCC to the date of death or last follow-up. Management was by a pre-agreed standardized multidisciplinary protocol supervised by a senior specialist surgeon. The ESCC cell line KYSE150 is of Japanese origin. It was purchased from DSMZ (Braunschweig, Germany) and cultured as described[17]. The non-tumor esophageal epithelial cell line NE1 was used as the control to confirm the overexpression of *GAEC1* in KYSE150 and was cultured as previously described[18].

Preparation of small interfering RNA expression vector

A vector based RNA interference (RNAi) approach was used for suppressing the expression of *GAEC1* in KYSE150 ESCC cells. The pSilencer2.1-U6 neo vector (Ambion) was used to express the siRNA which is specific for targeting *GAEC1* expression. The pSilencer2.1-U6 neo Negative Control vector (Ambion) was used as the negative control which expressed a hairpin small interfering RNA (siRNA) with limited homology to any known sequences in the human, mouse and rat genomes. The siRNA target sequence of *GAEC1*

and the insert sequence were determined by the programs siRNA Target Finder and Insert Design Tool for the pSilencer™ Vectors (Ambion). The top strand of the insert sequence (P3-4) is 5'-GATCCGAAGTGGCTTCTGGATTAATTCAAGAGATTAATCCAGAAGCCACTTCTTTTTTGGAAA-3' and the bottom strand is 5'-AGCTTTTCCAAAAAAGAAGTGGCTTCTGGATTAATCTCTTGAATTAATCCAGAAGCCACTTCG-3'. The top and bottom strands were annealed and cloned into the pSilencer2.1-U6 neo vector according to the manufacturer's instruction. The vectors were transfected into the KYSE150 cells as previously described[15] using FuGene HD (Roche Diagnostics GmbH) with G418 selection.

RNA extraction and reverse transcription-polymerase chain reaction analysis

RNA was extracted from the parental, pSilencer-P3-4 and pSilencer-negative control vectors transfected KYSE150 cells using the RNeasy mini Kit (Qiagen) after 2 mo selection under G418. About 2 μg DNA-free RNA from each sample was used for the multiplex semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis with β-actin as the internal control to show the expression level of *GAEC1* as previously described[15]. Densitometry analysis was performed to compare the intensity of the PCR products after agarose gel electrophoresis under UV using Quantity-One program (Bio-Rad).

Cell proliferation assay

The cell proliferation of the parental, pSilencer-P3-4 and pSilencer-negative control vectors transfected KYSE150 cells was determined by MTS assay using the CellTiter 96 Aqueous One Solution (Promega) as previously described[15].

Cell cycle analysis

The parental, pSilencer-P3-4 and pSilencer-negative control vectors transfected KYSE150 cells were resuspended in 500 L 1 × phosphate buffered saline and fixed with 500 L 70% ethanol. The cells were then suspended in 1 mL PI (20 μg/mL)/Triton X-100 (0.1% v/v) staining solution with RNase A (200 μg/mL) and then analyzed by the BD FACSCalibur flow cytometer. Different fractions of cell cycles were analyzed using the Modfit LT software (Verity Software House).

cDNA microarray analysis

The differentially expressed genes of the pSilencer-P3-4 vector transfected KYSE150 cells with suppressed *GAEC1* expression were identified using cDNA microarray analysis by making comparisons between the parental cells, pSilencer-negative control vectors transfected cells, and pcDNA3.1-*GAEC1* transfected cells with *GAEC1* overexpression[15]. The cDNA microarray analysis and the associated quality control using Human Genome U133 Plus 2.0 arrays (Affymetrix) were performed in the Genome Research Centre of the University of Hong Kong according to the Affymetrix's protocol. Briefly, total RNA was extracted from 2×10^6 cells of each sample using RNeasy mini Kit (Qiagen). The RNA integrity was measured by the ratio of 28S/18S ribosomal RNA using Agilent 2100 Bioanalyzer. One microgram total RNA from each source was then reverse transcribed to the first-stranded cDNA by using oligo-dT linked-T7 RNA polymerase promoter sequence and the double-stranded cDNA was synthesized by using RT Kit (Invitrogen). The biotin labelled-cRNA was produced by *in vitro* transcription kit (Invitrogen) and purified by RNeasy mini columns (Qiagen). About 15 μg denatured cRNA was hybridized to each Human Genome U133 Plus 2.0 array (Affymetrix) and then stained with a streptavidin-phycoerythrin conjugate and the signals were detected with GeneArray scanner (Agilent). The microarray signals were analyzed by using Agilent Genespring GX and Affymetrix GeneChip Operating Softwares. The signal of each differentially expressed gene in the pSilencer-P3-4 transfected cells was determined by comparing with the parental cells and normalized with the pSilencer-negative control vector transfected cells. The threshold level of the corresponding up- or down-regulated genes with transfected pcDNA3.1-*GAEC1* vector is ⩾ 2 folds.

Tissue microarray and immunohistochemical staining

A TMA containing the 132 paired non-tumor esophageal epithelia and ESCC specimens were constructed as described previously[19]. The archival paraffin-embedded ESCC tissues were used under the ethical guidelines in the Department of Pathology of The University of Hong Kong. Immunohisto-chemical staining on the TMA sections was performed using the calpain 10 (0.03 mg/mL; Sigma-Aldrich) rabbit polyclonal antibody and *TNRC6C* (1 mg/mL; Abnova) mouse monoclonal antibody using the previously described methodology[19]. The dilution factors for the calpain 10 and *TNRC6C* antibodies were 1:50 and 1:150 respectively. The percentage of tumor cells positively stained formed the basis of grading as follows: Grade 0: less than 5%, Grade Ⅰ: 5% to less than 25%, Grade Ⅱ: 25% to less than 50% and Grade Ⅲ: more than 50%. For each tissue sample, the tissue core with the highest grade was selected for subsequent statistical analysis. The high expression group combined those tumors with Grade Ⅱ or Ⅲ, and the low expression group combined those tumors with Grade 0 or Ⅰ.

Statistical analysis

The Student's *t* test was used to evaluate the statistical significance of the differences in calpain 10 and *TNRC6C* expression between tumor and non-tumor tissues. The χ^2 test and *t* test were used to examine the statistical significance of the correlations between calpain 10 and *TNRC6C* expression with clinicopathological parameters. Kaplan-Meier plots and Cox multi-variant analysis were produced for overall patient survival, and statistical significance was evaluated by using Wilcoxon's signed-rank test. Statistical analysis were performed using SPSS Ver. 20.0 (SPSS, Chicago, IL, United States). Differences were

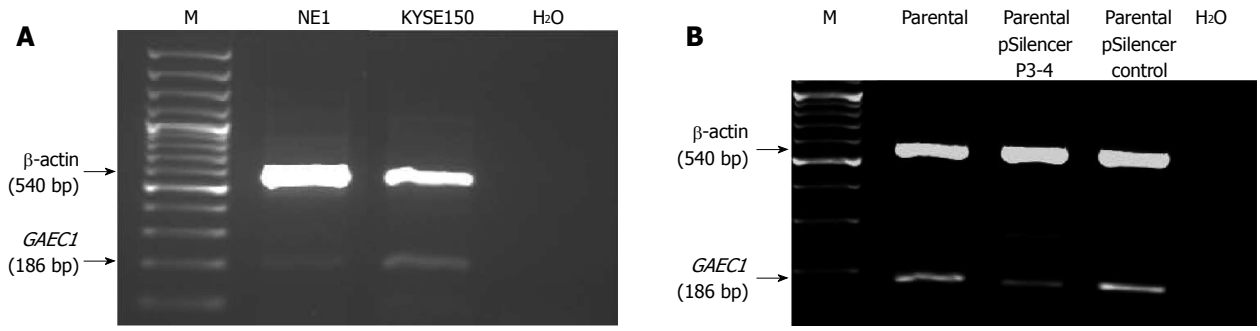

Figure 1 Expression level of *GAEC1* in KYSE150 cells. A: Multiplex reverse transcription-polymerase chain reaction (RT-PCR) analysis showed the overexpression of *GAEC1* in KYSE150 compared with the non-tumor esophageal epithelial cell line NE1; B: Multiplex RT-PCR analysis demonstrated the down-regulation of *GAEC1* expression in KYSE150 cells transfected with pSilencer P3-4 vector compared with the parental cells and those transfected with pSilencer control vector. The amount of RNA in each lane was normalized with the amplification of β-actin. M: 100 bp ladder marker; H_2O: Water control.

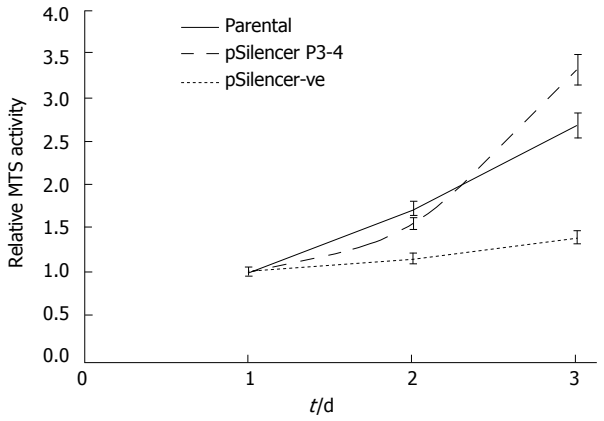

Figure 2 MTS cell proliferation assays for esophageal squamous cell carcinoma cell line KYSE150. Cells were transfected with pSilencer vector cloned with the P3-4 sequence (pSilencer P3-4) or control vector (pSilencer-ve). MTS assays were then performed every 24 h for 3 d on each type of transfected cells and the parental cells. The respective MTS activities on each day were compared with the corresponding activities of day 1. Representative data from 3 independent experiments are shown.

considered statistically significant when the relevant *P* values were < 0.05.

RESULTS

The overexpression of *GAEC1* in KYSE150 over NE1 was confirmed by multiplex semi-quantitative RT-PCR and densitometry analysis (Figure 1A). The expression level of *GAEC1* in pSilencer-P3-4 transfected KYSE150 cells was also determined by comparing with the parental and pSilencer-negative control vector transfected cells using densitometry measurement. The results indicated that the pSilencer-P3-4 transfected KYSE150 cells showed a down-regulation of *GAEC1* expression compared with the parental cells and the control. The comparison of the band intensities among the samples by densitometry measurement showed that the *GAEC1* expression level was down-regulated in the pSilencer-P3-4 transfected cells by about three folds (Figure 1B).

To assess the effect on cell proliferation with suppressed *GAEC1* expression, a comparison was made between the MTS activities generated from the parental cells and the cells transfected with the pSilencer-negative control vector. The results indicated that the KYSE150 cells with down-regulated *GAEC1* showed an obvious reduction in proliferation rate compared with the parental and control-vector transfected cells (Figure 2). Further analysis on the cell cycle related changes using flow cytometry demonstrated an approximately 50% increase in apoptotic population with suppressed *GAEC1* expression, compared with the parental and control-vector transfected cells (Figure 3).

To identify the downstream candidate genes which are regulated by the suppressed *GAEC1* expression, cDNA microarray analysis was performed using the Human Genome U133 Plus 2.0 array (Affymetrix) which comprises of more than 47000 transcripts and variants in each chip. The results of the identified lists of more than 5-fold down-regulated (total 10 genes) and more than 3-fold up-regulated (total 9 genes) targets were shown in Table 1 respectively. All the listed genes were selected based on more than 2-fold expression signals of the corresponding up- or down-regulation of the respective genes when the cells were transfected with the pcDNA3.1-*GAEC1* vector and no significant fold change was detected with transfected pSilencer-negative control vector compared with parental cells. With suppressed *GAEC1* expression, calpain 10 (*CAPN10*) was identified to have the highest level (> 15 folds) of down-regulation (Table 1), while trinucleotide repeat containing 6C (*TNRC6C*) was shown to have the highest level (> 7 folds) of up-regulation (Table 1). These two *GAEC1*-regulated target genes with the greatest changes in expression level were followed up by the immunohistochemical analysis using the ESCC tissue microarray.

The expression of *CAPN10* and *TNRC6C* proteins in TMA sections sampling 132 paired tumor and non-tumor tissues from ESCC specimens was investigated using immunohistochemistry. Fourteen out of 132 tumors (10.61%) were found to belong to the high expression group of *CAPN10* expression. However, *TNRC6C* did not show any significant expression signals in all the ESCC cases analyzed except eight non-tumor esopha-

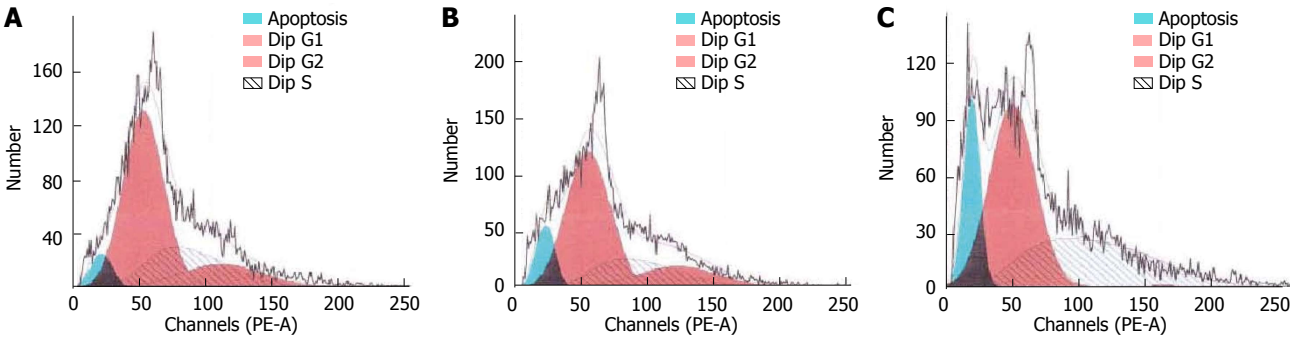

Figure 3 Flow cytometry analyses for KYSE150 cells. KYSE150 transfected with pSilencer cloned with P3-4 sequence demonstrated an increased apoptotic population by approximately 50% (C) compared with the parental cells (A) and cells transfected with pSilencer-ve control vector (B).

Table 1 List of more than 5-fold and 3-fold down-regulated genes induced by stable *GAEC1* knowndown in KYSE150 cells compared with the parental cells

Probe set ID	Gene title	Down-regulation with transfected pSilencer P3-4	Up-regulation with transfected pcDNA3.1-*GAEC1*	pSilencer-ve control
221040_at	Calpain 10	15.3033010	2.1643467	1.0476209
1561417_x_at	Not assigned	12.1628650	2.0130675	1.1347373
1562828_at	Not assigned	9.9816000	2.6808436	1.1661105
229929_at	splA/ryanodine receptor domain and SOCS box containing 4	8.3420770	2.2365010	1.1751518
235209_at	Chromosome 8 open reading frame 84	7.6294910	2.1356385	1.1450081
220090_at	Cornulin	7.6125007	2.3401918	1.1664450
242713_at	Not assigned	7.3507795	2.0578532	1.1959343
224499_s_at	Activation-induced cytidine deaminase	5.8917794	3.3146940	1.1664389
229543_at	Not assigned	5.3493247	2.0206234	1.0065930
242064_at	Sidekick homolog 2 (chicken)	5.0322995	2.8469403	1.0170712
1561041_at	Trinucleotide repeat containing 6C	7.5979643	2.1234870	1.0152589
216787_at	Not assigned	5.3369575	2.2657390	1.1658608
206725_x_at	Bone morphogenetic protein 1	4.8828310	2.7046654	1.1015952
206276_at	Lymphocyte antigen 6 complex, locus D	4.7652740	2.7379642	1.0686288
1560482_at	Not assigned	4.2348604	3.0122058	1.1875614
211362_s_at	Serpin peptidase inhibitor, clade B (ovalbumin), member 13	4.0584164	2.4208739	1.0186443
216491_x_at	Immunoglobulin heavy constant mu	3.4819565	2.8060850	1.0186309
238415_at	Not assigned	3.2034543	2.9523630	1.0138865
241028_at	RPGRIP1-like	3.0331728	2.3752263	1.0001514

All the listed genes were selected based on more than 2-fold of the corresponding up-regulation when *GAEC1* was overexpressed with transfected pcDNA3.1-*GAEC1* vector and no significant fold change with transfected pSilencer-negative control vector compared with parental cells.

geal tissues which also served as the positive controls. Representative examples of immunohistochemical staining of *CAPN10* and *TNRC6C* are shown in Figure 4. Correlation between expression level of *CAPN10* and clinicopathological features are summarized in Table 2. There was no significant correlation of any clinicopathological features with the expression level of *CAPN10*. The median survival of patients with high expression level of *CAPN10* was 38 mo whereas that of low expression level was 13 mo, and the survival range is from 0.72 to 65.15 mo. The difference was significant on both univariant and multi-variant analysis ($P = 0.032$ and 0.035 respectively; Figure 5).

DISCUSSION

Our previous study reported the oncogenic role of *GAEC1* in esophageal carcinogenesis and high expression level of *GAEC1* caused malignant transformation of normal cells[15]. High DNA copy number of *GAEC1* was also observed in colorectal adenocarcinoma and significant difference was reported in cancer sub-sites and tumor types[16]. From our previous study[15], however, no significant correlation was observed between *GAEC1* amplification and clinicopathological parameters and prognosis in ESCC tumors, and thus the DNA amplification study of *GAEC1* is not included in this report. In the present study, an attempt was made to investigate the downstream- regulated genes when *GAEC1* expression was suppressed in an ESCC cell line KYSE150. Our group also investigated the ESCC cell lines which showed overexpression of *GAEC1* as we reported previously[15]. KYSE150 showed the more stable and consistent overexpression with time compared among the ESCC cell lines. Reduction of proliferation rate and increase in apoptotic population were observed in association with reduced

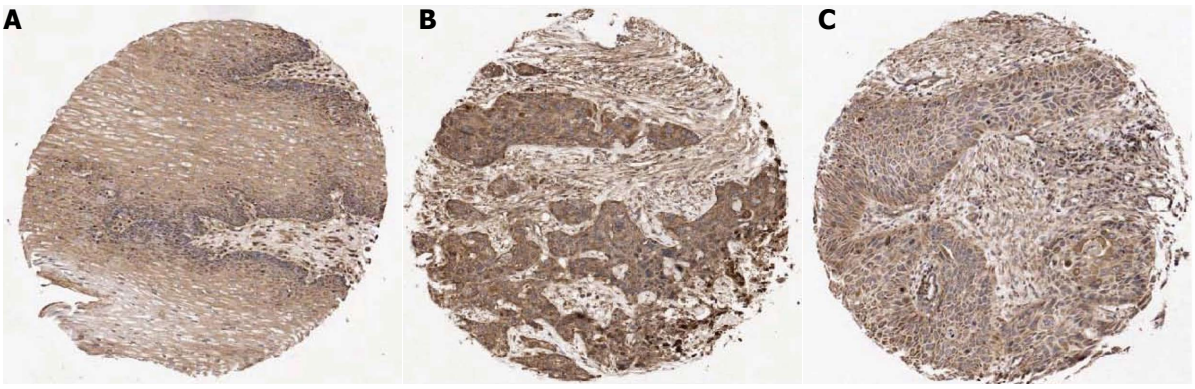

Figure 4 Immunohistochemical staining of *CAPN10*. A: In normal esophageal epithelial tissue showing weak *CAPN10* staining; B: esophageal squamous cell carcinoma (ESCC) tissue showing strong *CAPN10* staining; C: ESCC tissue showing weak *CAPN10* staining in tumor. *CAPN10* was mainly localized in the cytoplasm of the cancer cells (original magnification, ×100).

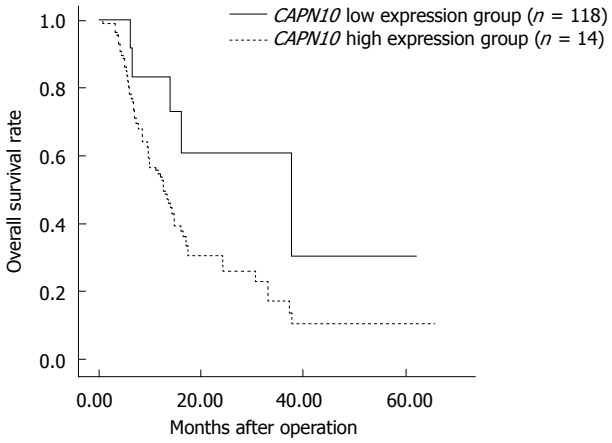

Figure 5 Overall 5-year survival rates as determined by the expression level of *CAPN10* in esophageal squamous cell carcinoma patients. Low expression group of *CAPN10* in ESCC patients showed a significantly lower 5-year survival rate than those of high expression group.

Table 2 Relationship between *CAPN10* expression and clinicopathological features

Characteristics	Patients	Low expression	High expression	*P* value
Age, yr (mean ± SD)	132	65.64 ± 10.55	63.50 ± 13.39	0.572
Gender				0.086
Male	102	94	8	
Female	30	24	6	
TNM stage				0.762
0/ I / II	18	15	9	
III / IV	83	72	11	
Tumor depth				0.729
T1-3	79	67	12	
T4	22	20	2	
Lymph node metastasis				0.363
N0	32	26	6	
N1	69	61	8	
Distant metastasis				1
M0	66	57	9	
M1	35	30	5	
Differentiation				0.459
Well	16	15	1	
Moderate	59	51	8	
Poor	26	21	5	

GAEC1 expression in ESCC cells. Thus our study is the first report to demonstrate the significance of suppressing *GAEC1* as a target of reducing the malignant properties of ESCC. In order to assess whether the tumors are more proliferative, the use of other histological markers for assessing proliferation, such as Ki-67[20] and AgNOR[21], in parallel to *CAPN10* is suggested in future studies to determine whether the *CAPN10* level is associated with progression of the disease. Similar targeting approach against potential oncogenes is now being explored intensively in the direction of gene therapy for various types of cancers[22]. Examples include the suppression of *MTA1* in esophageal carcinoma[23], alpha-actinin-4 in oral carcinoma[24], osteopontin in colon carcinoma[25] and *EGFR* in hepatocellular carcinoma[26]. The application of RNAi approach has been recognized as having high potential for the clinical application of targeted cancer therapy[27]. To date, clinical trials at different stages were reported and they targeted against various oncogenic components in various cancers, including metastatic melanoma, liver cancer, chronic myelogenous leukemia, pancreatic cancer and colon cancer[22]. Moreover, the RNAi approach for targeting specifically on transforming growth factor-β has been developed as a "cancer vaccine" against ovarian cancer[22]. Thus our present study offers a new direction for exploring the application of RNAi-based method for suppressing the oncogenic target *GAEC1* as a novel gene therapy approach in our future investigations.

Calpain 10 (*CAPN10*) is a member of the mitochondrial calpain system[28]. Mitochondrial calpain system has been shown to promote caspase-independent programmed cell death *via* the apoptotic inducing factor-mediated mechanism[28] and its expression has been correlated to insulin-stimulated glucose uptake[29] and type 2 diabetes[30]. However, the correlation and functional roles of *CAPN10* in tumorigenesis are still not fully understood, although *CAPN10* has been linked to laryngeal[31], colorectal[32] and pancreatic cancers[33]. In the present study, the RNAi-based suppression of *GAEC1*

in KYSE150 resulted in the suppression of *CAPN10* expression (approximately 15-fold compared with the parental cells). For those ESCC tumors belonging to the *CAPN10* low expression group, the 5-year survival rate is significantly lower than those belonging to the *CAPN10* high expression group. From the study of Moreno-Luna *et al*[31], *CAPN10* genotype 12 was reported to be related with a worse prognosis in laryngeal cancer, which is similar to our present study which is newly described in ESCC. Our observation from the low *CAPN10* expression group implied the possibility that the oncogene *GAEC1* overexpression within this group might involve more prominently at the initial stage of molecular carcinogenesis, so that the expression level of *CAPN10* was lower in ESCC at the time of operation. Similar pattern of oncogenic expression happening at the earlier stage of carcinogenesis was also observed from fibroblast growth factor-2 in melanoma[34] and *KLF4* in cutaneous squamous epithelial neoplasia[35]. The verification of this hypothesis can be followed up with the future development of *GAEC1*-specific antibody, which is still unavailable in market, for the future analysis of *GAEC1* expression in various stages in ESCC. This important finding also paves the path for the further investigation for the roles of *CAPN10* in the molecular pathogenesis of esophageal carcinoma. Moreover, in the present study, no significant correlation of any other clinicopathological features with the expression level of *CAPN10* was found, but *CAPN10* predicted the poor survival of ESCC patients. Similar results were also reported previously in which the overexpression of a chemokine *CXCL12* in ovarian cancer[36] and a protein Rad51 for homologous recombination in ESCC[37] also showed a correlation to the survival of patients, but no correlation to other clinicopathological features was found. The level of *CAPN10* is also not associated with local lymph node and distant metastasis in the ESCC cases, implying the possibility that *GAEC1* expression may not be relevant to the control of metastasis in ESCC.

TNRC6C has been reported to be the miRNA regulation-related genes and their mutation was correlated to cancer development through deregulating the miRNA regulation[38]. *TNRC6C* was also shown in the present study to undergo up-regulation with the suppression of *GAEC1* expression by the RNAi approach, but there was no significant expression of *TNRC6C* in the ESCC cases studied. This may be due to the down-regulation of *TNRC6C* in ESCC by other unknown mechanisms which are subjected to further investigation. Future study of *TNRC6C* mutation in ESCC is required to investigate the possible roles of *TNRC6C* in carcinogenesis. Moreover, among the down-regulated genes identified by cDNA microarray with suppressed *GAEC1* expression, activation-induced cytidine deaminase (AID) was reported to show overexpression in Barrett's esophagus and Barrett's adenocarcinoma[39], but there was no investigation on the roles of AID in molecular pathogenesis in ESCC. AID has been shown to induce somatic mutations in host genes and implicated in the carcinogenesis of lung[40], colorectal[41] and gastric cancers[42]. Therefore, our findings provide a new evidence for prompting future study on the role of AID in the development of ESCC.

In conclusion, the suppression of *GAEC1* expression resulted in reduced tumor cell proliferation, increased apoptotic population in ESCC cells and also regulated *CAPN10* and *TNRC6C* expression. The low expression of *CAPN10* predicted the poor survival of ESCC patients.

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COMMENTS

Background

Esophageal squamous cell carcinoma (ESCC) has a multifactorial etiology which involves environmental and/or genetic factors. More investigations at the molecular level are essential for a better understanding the molecular pathogenesis of this disease. Authors subsequently identified and characterized the role of a novel oncogene *GAEC1* which is located at 7q22 region. In this report, *GAEC1* was further characterized by identifying the downstream partners. The prominently downstream-regulated genes were then studied by immunohistochemistry on ESCC tissues to determine their clinicopathological significance.

Research frontiers

The anti-proliferative effect of knocking-down the expression of *GAEC1* in ESCC cells was studied. The research hotspot is to find out the target genes which are most regulated by *GAEC1* and to determine their clinicopathological significance in ESCC.

Innovations and breakthroughs

The RNA interference (RNAi) approach showed effective suppression of *GAEC1* expression in ESCC cells to inhibit cell proliferation and increase apoptosis. cDNA microarray analysis for differentially expressed genes identified the greatest levels of downregulation of calpain 10 (*CAPN10*) and upregulation of trinucleotide repeat containing 6C when *GAEC1* expression was suppressed. High level expression of calpain 10 was significantly associated with longer patient survival. This is the first study to explore the regulatory roles of *GAEC1* on the downstream targets and to report the association of *CAPN10* to the survival of ESCC patients.

Applications

This study suggested that the potential use of *CAPN10* as a prognostic marker to predict the survival of ESCC patients after operation. The findings of the present study pave the path for the future related studies in other human cancers.

Terminology

Squamous cell carcinoma: It is a cancer of a kind of epithelial cell called squamous cell. Squamous cells also occur in the lining of the digestive tract, such as the esophagus; Oncogene: An oncogene is a gene that has the potential to cause cancer. In tumor cells, they are often mutated or expressed at high levels.

Peer review

This is a good study in which the authors employed *GAEC1* RNAi to knockdown the expression of *GAEC1*, investigated its effects on *GAEC1*-overexpressed esophageal carcinoma cell line KYSE150, and then explored the possible mechanisms. The study design is reasonable, statistical methods are appropriate.

REFERENCES

1 **Wang JM**, Xu B, Rao JY, Shen HB, Xue HC, Jiang QW. Diet habits, alcohol drinking, tobacco smoking, green tea drinking, and the risk of esophageal squamous cell

carcinoma in the Chinese population. *Eur J Gastroenterol Hepatol* 2007; **19**: 171-176 [PMID: 17273005 DOI: 10.1097/MEG.0b013e32800ff77a]
2 **Lam AK**. Molecular biology of esophageal squamous cell carcinoma. *Crit Rev Oncol Hematol* 2000; **33**: 71-90 [PMID: 10737369 DOI: 10.1016/s1040-8428(99)00054-2]
3 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]
4 **Ghadimi MR**, Rasouli M, Mahmoodi M, Mohammad K. Prognostic factors for the survival of patients with esophageal cancer in Northern Iran. *J Res Med Sci* 2011; **16**: 1261-1272 [PMID: 22973319]
5 **Bellini MF**, Silva AE, Varella-Garcia M. Genomic imbalances in esophageal squamous cell carcinoma identified by molecular cytogenetic techniques. *Genet Mol Biol* 2010; **33**: 205-213 [PMID: 21637470 DOI: 10.1590/s1415-47572010005000028]
6 **Kuwano H**, Kato H, Miyazaki T, Fukuchi M, Masuda N, Nakajima M, Fukai Y, Sohda M, Kimura H, Faried A. Genetic alterations in esophageal cancer. *Surg Today* 2005; **35**: 7-18 [PMID: 15622457 DOI: 10.1007/s00595-004-2885-3]
7 **Tang JC**, Lam KY, Law S, Wong J, Srivastava G. Detection of genetic alterations in esophageal squamous cell carcinomas and adjacent normal epithelia by comparative DNA fingerprinting using inter-simple sequence repeat PCR. *Clin Cancer Res* 2001; **7**: 1539-1545 [PMID: 11410488]
8 **Touchman JW**, Bouffard GG, Weintraub LA, Idol JR, Wang L, Robbins CM, Nussbaum JC, Lovett M, Green ED. 2006 expressed-sequence tags derived from human chromosome 7-enriched cDNA libraries. *Genome Res* 1997; **7**: 281-292 [PMID: 9074931 DOI: 10.1101/gr.7.3.281]
9 **Glöckner G**, Scherer S, Schattevoy R, Boright A, Weber J, Tsui LC, Rosenthal A. Large-scale sequencing of two regions in human chromosome 7q22: analysis of 650 kb of genomic sequence around the EPO and CUTL1 loci reveals 17 genes. *Genome Res* 1998; **8**: 1060-1073 [PMID: 9799793]
10 **Schaefer IM**, Enders C, Polten A, Haller F, Frölich AM, Cameron S, Schüler P, Schweiger P, Gunawan B, Beham A, Füzesi L. Common genomic aberrations in basaloid squamous cell carcinoma and carcinosarcoma of the esophagus detected by CGH and array CGH. *Am J Clin Pathol* 2011; **135**: 579-586 [PMID: 21411780 DOI: 10.1309/ajcpz1o7uuuispnr]
11 **Pinto AE**, Roque L, Rodrigues R, André S, Soares J. Frequent 7q gains in flow cytometric multiploid/hypertetraploid breast carcinomas: a study of chromosome imbalances by comparative genomic hybridisation. *J Clin Pathol* 2006; **59**: 367-372 [PMID: 16461572 DOI: 10.1136/jcp.2005.027722]
12 **Chang MC**, Chang YT, Tien YW, Sun CT, Wu MS, Lin JT. Distinct chromosomal aberrations of ampulla of Vater and pancreatic head cancers detected by laser capture microdissection and comparative genomic hybridization. *Oncol Rep* 2005; **14**: 867-872 [PMID: 16142344]
13 **Baudis M**. Genomic imbalances in 5918 malignant epithelial tumors: an explorative meta-analysis of chromosomal CGH data. *BMC Cancer* 2007; **7**: 226 [PMID: 18088415 DOI: 10.1186/1471-2407-7-226]
14 **Miyata T**, Yonekura K, Utsunomiya A, Kanekura T, Nakamura S, Seto M. Cutaneous type adult T-cell leukemia/lymphoma is a characteristic subtype and includes erythema/papule and nodule/tumor subgroups. *Int J Cancer* 2010; **126**: 1521-1528 [PMID: 19739121 DOI: 10.1002/ijc.24874]
15 **Law FB**, Chen YW, Wong KY, Ying J, Tao Q, Langford C, Lee PY, Law S, Cheung RW, Chui CH, Tsao SW, Lam KY, Wong J, Srivastava G, Tang JC. Identification of a novel tumor transforming gene GAEC1 at 7q22 which encodes a nuclear protein and is frequently amplified and overexpressed in esophageal squamous cell carcinoma. *Oncogene* 2007; **26**: 5877-5888 [PMID: 17384685 DOI: 10.1038/sj.onc.1210390]
16 **Gopalan V**, Smith RA, Nassiri MR, Yasuda K, Salajegheh A, Kim SY, Ho YH, Weinstein S, Tang JC, Lam AK. GAEC1 and colorectal cancer: a study of the relationships between a novel oncogene and clinicopathologic features. *Hum Pathol* 2010; **41**: 1009-1015 [PMID: 20236690 DOI: 10.1016/j.humpath.2009.11.014]
17 **Shimada Y**, Imamura M, Wagata T, Yamaguchi N, Tobe T. Characterization of 21 newly established esophageal cancer cell lines. *Cancer* 1992; **69**: 277-284 [PMID: 1728357 DOI: 10.1002/1097-0142(19920115)69]
18 **Deng W**, Tsao SW, Guan XY, Lucas JN, Si HX, Leung CS, Mak P, Wang LD, Cheung AL. Distinct profiles of critically short telomeres are a key determinant of different chromosome aberrations in immortalized human cells: whole-genome evidence from multiple cell lines. *Oncogene* 2004; **23**: 9090-9101 [PMID: 15489894 DOI: 10.1038/sj.onc.1208119]
19 **Yuen HF**, Chan YP, Chan KK, Chu YY, Wong ML, Law SY, Srivastava G, Wong YC, Wang X, Chan KW. Id-1 and Id-2 are markers for metastasis and prognosis in oesophageal squamous cell carcinoma. *Br J Cancer* 2007; **97**: 1409-1415 [PMID: 18000500 DOI: 10.1038/sj.bjc.6604035]
20 **Brown DC**, Gatter KC. Ki67 protein: the immaculate deception? *Histopathology* 2002; **40**: 2-11 [PMID: 11903593 DOI: 10.1046/j.1365-2559.2002.01343.x]
21 **Derenzini M**. The AgNORs. *Micron* 2000; **31**: 117-120 [PMID: 10588056 DOI: 10.1016/s0968-4328(99)00067-0]
22 **Burnett JC**, Rossi JJ, Tiemann K. Current progress of siRNA/shRNA therapeutics in clinical trials. *Biotechnol J* 2011; **6**: 1130-1146 [PMID: 21744502 DOI: 10.1002/biot.201100054]
23 **Qian H**, Lu N, Xue L, Liang X, Zhang X, Fu M, Xie Y, Zhan Q, Liu Z, Lin C. Reduced MTA1 expression by RNAi inhibits in vitro invasion and migration of esophageal squamous cell carcinoma cell line. *Clin Exp Metastasis* 2005; **22**: 653-662 [PMID: 16703414 DOI: 10.1007/s10585-006-9005-2]
24 **Yamada S**, Yanamoto S, Yoshida H, Yoshitomi I, Kawasaki G, Mizuno A, Nemoto TK. RNAi-mediated down-regulation of alpha-actinin-4 decreases invasion potential in oral squamous cell carcinoma. *Int J Oral Maxillofac Surg* 2010; **39**: 61-67 [PMID: 19913389 DOI: 10.1016/j.ijom.2009.10.003]
25 **Likui W**, Hong W, Shuwen Z, Yuangang Y, Yan W. The potential of osteopontin as a therapeutic target for human colorectal cancer. *J Gastrointest Surg* 2011; **15**: 652-659 [PMID: 21318445 DOI: 10.1007/s11605-011-1445-6]
26 **Hu Y**, Shen Y, Ji B, Wang L, Zhang Z, Zhang Y. Combinational RNAi gene therapy of hepatocellular carcinoma by targeting human EGFR and TERT. *Eur J Pharm Sci* 2011; **42**: 387-391 [PMID: 21238587]
27 **Vázquez-Vega S**, Contreras-Paredes A, Lizano-Soberón M, Amador-Molina A, García-Carrancá A, Sánchez-Suárez LP, Benítez-Bribiesca L. [RNA interference (RNAi) and its therapeutic potential in cancer]. *Rev Invest Clin* 2010; **62**: 81-90 [PMID: 20415062]
28 **Kar P**, Samanta K, Shaikh S, Chowdhury A, Chakraborti T, Chakraborti S. Mitochondrial calpain system: an overview. *Arch Biochem Biophys* 2010; **495**: 1-7 [PMID: 20035707 DOI: 10.1016/j.abb.2009.12.020]
29 **Brown AE**, Yeaman SJ, Walker M. Targeted suppression of calpain-10 expression impairs insulin-stimulated glucose uptake in cultured primary human skeletal muscle cells. *Mol Genet Metab* 2007; **91**: 318-324 [PMID: 17572128 DOI: 10.1016/j.ymgme.2007.05.001]
30 **Horikawa Y**. Calpain-10 (NIDDM1) as a Susceptibility Gene for Common Type 2 Diabetes. *Endocr J* 2006; **53**: 567-576 [PMID: 16873988 DOI: 10.1507/endocrj.KR-70]
31 **Moreno-Luna R**, Abrante A, Esteban F, González-Moles MA, Delgado-Rodríguez M, Sáez ME, González-Pérez A, Ramírez-Lorca R, Real LM, Ruiz A. Calpain 10 gene and laryngeal cancer: a survival analysis. *Head Neck* 2011; **33**: 72-76 [PMID: 20848425 DOI: 10.1002/hed.21404]
32 **Frances CP**, Conde MC, Saez ME, Diez SF, Rey CM, Ramírez-Armengol JA, Pascual MH, Gonzalez-Perez A, Torres PP, Real LM, Serrano-Rios M, López JL, Ruiz A, Royo JL. Identi-

fication of a protective haplogenotype within CAPN10 gene influencing colorectal cancer susceptibility. *J Gastroenterol Hepatol* 2007; **22**: 2298-2302 [PMID: 17559371 DOI: 10.1111/j.1440-1746.2007.04843.x]

33 **Fong PY**, Fesinmeyer MD, White E, Farin FM, Srinouanprachanh S, Afsharinejad Z, Mandelson MT, Brentnall TA, Barnett MJ, Goodman GE, Austin MA. Association of diabetes susceptibility gene calpain-10 with pancreatic cancer among smokers. *J Gastrointest Cancer* 2010; **41**: 203-208 [PMID: 20178008 DOI: 10.1007/s12029-010-9130-7]

34 **Tsunoda S**, Nakamura T, Sakurai H, Saiki I. Fibroblast growth factor-2-induced host stroma reaction during initial tumor growth promotes progression of mouse melanoma via vascular endothelial growth factor A-dependent neovascularization. *Cancer Sci* 2007; **98**: 541-548 [PMID: 17359289 DOI: 10.1111/j.1349-7006.2007.00432.x]

35 **Huang CC**, Liu Z, Li X, Bailey SK, Nail CD, Foster KW, Frost AR, Ruppert JM, Lobo-Ruppert SM. KLF4 and PCNA identify stages of tumor initiation in a conditional model of cutaneous squamous epithelial neoplasia. *Cancer Biol Ther* 2005; **4**: 1401-1408 [PMID: 16357510]

36 **Popple A**, Durrant LG, Spendlove I, Rolland P, Scott IV, Deen S, Ramage JM. The chemokine, CXCL12, is an independent predictor of poor survival in ovarian cancer. *Br J Cancer* 2012; **106**: 1306-1313 [PMID: 22415233 DOI: 10.1038/bjc.2012.49]

37 **Li Y**, Yu H, Luo RZ, Zhang Y, Zhang MF, Wang X, Jia WH. Elevated expression of Rad51 is correlated with decreased survival in resectable esophageal squamous cell carcinoma. *J Surg Oncol* 2011; **104**: 617-622 [PMID: 21744352 DOI: 10.1002/jso.22018]

38 **Kim MS**, Oh JE, Kim YR, Park SW, Kang MR, Kim SS, Ahn CH, Yoo NJ, Lee SH. Somatic mutations and losses of expression of microRNA regulation-related genes AGO2 and TNRC6A in gastric and colorectal cancers. *J Pathol* 2010; **221**: 139-146 [PMID: 20198652 DOI: 10.1002/path.2683]

39 **Morita S**, Matsumoto Y, Okuyama S, Ono K, Kitamura Y, Tomori A, Oyama T, Amano Y, Kinoshita Y, Chiba T, Marusawa H. Bile acid-induced expression of activation-induced cytidine deaminase during the development of Barrett's oesophageal adenocarcinoma. *Carcinogenesis* 2011; **32**: 1706-1712 [PMID: 21890457 DOI: 10.1093/carcin/bgr194]

40 **Shinmura K**, Igarashi H, Goto M, Tao H, Yamada H, Matsuura S, Tajima M, Matsuda T, Yamane A, Funai K, Tanahashi M, Niwa H, Ogawa H, Sugimura H. Aberrant expression and mutation-inducing activity of AID in human lung cancer. *Ann Surg Oncol* 2011; **18**: 2084-2092 [PMID: 21290192 DOI: 10.1245/s10434-011-1568-8]

41 **Endo Y**, Marusawa H, Chiba T. Involvement of activation-induced cytidine deaminase in the development of colitis-associated colorectal cancers. *J Gastroenterol* 2011; **46** (Suppl 1): 6-10 [PMID: 20878190 DOI: 10.1007/s00535-010-0326-1]

42 **Goto A**, Hirahashi M, Osada M, Nakamura K, Yao T, Tsuneyoshi M, Takayanagi R, Oda Y. Aberrant activation-induced cytidine deaminase expression is associated with mucosal intestinalization in the early stage of gastric cancer. *Virchows Arch* 2011; **458**: 717-724 [PMID: 21538122 DOI: 10.1007/s00428-011-1086-x]

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ORIGINAL ARTICLE

MAWBP and MAWD inhibit proliferation and invasion in gastric cancer

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Abstract

AIM: To investigate role of putative mitogen-activated protein kinase activator with WD40 repeats (MAWD)/MAWD binding protein (MAWBP) in gastric cancer (GC).

METHODS: MAWBP and MAWD mRNA expression level was examined by real-time reverse transcriptase-polymerase chain reaction and semi-quantitative polymerase chain reaction in six GC cell lines. Western blotting was used to examine the protein expression levels. We developed GC cells that stably overexpressed MAWBP and MAWD, and downregulated expression by RNA interference assay. Proliferation and migration of these GC cells were analyzed by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT), soft agar, tumorigenicity, migration and transwell assays. The effect of expression of MAWBP and MAWD on transforming growth factor (TGF)-β1-induced epithelial-mesenchymal transition (EMT) was examined by transfection of MAWBP and MAWD into GC cells. We detected the levels of EMT markers E-cadherin, N-cadherin and Snail in GC cells overexpressing MAWBP and MAWD by Western blotting. The effect of MAWBP and MAWD on TGF-β signal was detected by analysis of phosphorylation level and nuclear translocation of Smad3 using Western blotting and immunofluorescence.

RESULTS: Among the GC cell lines, expression of endogenous MAWBP and MAWD was lowest in SGC7901 cells and highest in BGC823 cells. MAWBP and MAWD were stably overexpressed in SGC7901 cells and knocked down in BGC823 cells. MAWBP and MAWD inhibited GC cell proliferation *in vitro* and *in vivo*. MTT assay showed that overexpression of MAWBP and MAWD suppressed growth of SGC7901 cells ($P < 0.001$), while knockdown of these genes promoted growth of BGC823 cells ($P < 0.001$). Soft agar colony formation experiments showed that overexpression of MAWBP and MAWD alone or together reduced colony formation compared with vector group in SGC7901 (86.25 ± 8.43, 12.75 ± 4.49, 30 ± 6.41 *vs* 336.75 ± 22.55, $P < 0.001$), and knocked-down MAWBP and MAWD demonstrated opposite effects (131.25 ± 16.54, 88.75 ± 11.12, 341.75 ± 22.23 *vs* 30.25 ± 8.07, $P < 0.001$). Tumorigenicity experiments revealed that overexpressed MAWBP and MAWD inhibited GC cell proliferation *in vivo* ($P < 0.001$). MAWBP and MAWD also inhibited GC cell invasion. Transwell assay showed that the number of traverse cells of MAWBP, MAWD and coexpression group were more than that in vector group (84 ± 16.57, 98.33 ± 9.8, 29 ± 16.39 *vs* 298 ± 11.86, $P < 0.001$). Coexpression of MAWBP and MAWD significantly decreased the cells traversing the matrix membrane. Conversely, knocked-down MAWBP

and MAWD correspondingly promoted invasion of GC cells (100.67 ± 14.57, 72.66 ± 8.51, 330.67 ± 20.55 *vs* 27 ± 11.53, $P < 0.001$). More importantly, coexpression of MAWBP and MAWD promoted EMT. Cells that coexpressed MAWBP and MAWD displayed a pebble-like shape and tight cell-cell adhesion, while vector cells showed a classical mesenchymal phenotype. Western blotting showed that expression of E-cadherin was increased, and expression of N-cadherin and Snail was decreased when cells coexpressed MAWBP and MAWD and were treated with TGF-β1. Nuclear translocation of p-Smad3 was reduced by attenuating its phosphorylation.

CONCLUSION: Coexpression of MAWBP and MAWD inhibited EMT, and EMT-aided malignant cell progression was suppressed.

Key words: Gastric cancer; Mitogen-activated protein kinase activator with WD40 repeats binding protein; Mitogen-activated protein kinase activator with WD40 repeats; Invasion; Transforming growth factor-β; Epithelial-mesenchymal transition

Core tip: Our previous study revealed that mitogen-activated protein kinase activator with WD40 repeats (MAWD) and MAWD binding protein (MAWBP), acting as a complex, were differentially expressed in gastric cancer (GC) tissues compared with that in normal gastric tissues. The present study provided direct evidence that MAWBP and MAWD inhibited proliferation and migration of GC cells. Importantly, interaction of MAWBP and MAWD influenced expression of epithelial-mesenchymal transition (EMT) markers induced by transforming growth factor (TGF)-β1 in GC cells. It indicated that coexpression of MAWBP and MAWD inhibited TGF-β1-induced EMT, thus suppressing EMT-aided GC malignant progression.

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INTRODUCTION

Gastric cancer (GC) is the second most common cause of cancer death worldwide and is especially common in China[1]. Multiple factors are involved in the development of GC carcinogenesis. Molecular genetic studies have addressed accumulation of multiple genes and proteins alteration involved in GC development[2,3]. Investigation of GC biomarkers has focused on discovering differential protein signatures and has explored their biology mechanisms. The genes involved include activation of *c-myc*, *erbB-2*, *c-met*, and *k-ras*[4-7] oncogenes and inactivation of tumor suppressor genes *p53*, *APC*, *E-cadherin* and *RUNX3*[8-10].

Our laboratory previously found (using 2D gel electrophoresis and mass spectrometry) that expression of mitogen-activated protein kinase activator with WD40 repeats (MAWD) and MAWD binding protein (MAWBP) were differentially expressed in GC tissues. MAWD interacts with MAWBP and forms complexes in GC cell lines[11], which suggests that these proteins are involved in GC carcinogenesis. Combined analysis of MAWBP and MAWD expression would provide useful information in uncovering their roles in GC.

The proteins MAWBP and MAWD were discovered in 2000 and 2001, respectively[12,13]. MAWD is widely expressed in many tissues and sequence analysis has indicated that MAWD contains a WD40 repeat domain. Datta *et al*[14] have shown that MAWD-homolog protein serine-threonine kinase receptor-associated protein (STRAP) recruits Smad7, forming a complex that increases inhibition of transforming growth factor (TGF)-β signaling. Iriyama *et al*[13] tried to detect MAWD-related protein using a conventional two-hybrid technique and found MAWBP had an affinity for MAWD. The effects of MAWD in cancer have been reported in breast, colon and lung cancer but views about its role in cancer are divergent[15]. However, there is no current report on the function of MAWD in GC, and little is known about MAWBP other than its affinity for MAWD.

We hypothesize that MAWBP and MAWD interactions have a key role in GC tumorigenesis, and therefore investigated their biological function in GC cell lines. We found that these two proteins inhibited cell proliferation, and coexpression of MAWBP and MAWD obviously suppressed migration as well as invasive behavior of GC cells. Recent evidence implies that epithelial-mesenchymal transition (EMT) contributes to cancer progression, invasion and metastasis in various cancers[16,17]. TGF-β is the main and best-characterized inducer of EMT during embryogenesis and cancer pathogenesis[18]. MAWBP and MAWD are involved in the TGF-β signaling pathway[14]. We further sought to determine whether coexpression of MAWBP and MAWD could inhibit TGF-β1-induced EMT.

The canonical EMT program is characterized by complex proteome changes, leading to loss of epithelial markers such as E-cadherin, and expression of mesenchymal markers such as vimentin and N-cadherin[19]. Transcriptional regulator Snail is also activated in EMT. TGF-β signaling regulates expression of Snail, SOX2 and SOX4[20].

In this study, we analyzed the effect of MAWBP and MAWD on expression of E-cadherin, N-cadherin and Snail. We further demonstrated the relationship of this effect with TGF-β signalling pathway via detection of the phosphorylation level and nuclear translocation of Smad3. Our findings suggest that coexpression of MAWBP and MAWD inhibits TGF-β1-induced EMT

and suppresses EMT-aided GC cell invasion.

MATERIALS AND METHODS

Cell lines and cell culture

GC cell lines BGC823, MGC803, SGC7901, AGS, N87 and MKN45 were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco BRL, Gaithersburg, MD, United States), supplemented with 5% fetal bovine serum (FBS). All cell lines were maintained at 37 ℃ in 5% CO_2 as previously described[21,22].

Plasmid construction

We constructed MAWBP and MAWD expression plasmids using pcDNA3.1B(-). Total RNA was extracted from 19-wk-old fetal liver. MAWBP and MAWD cDNA was produced by reverse-transcriptase polymerase chain reaction (RT-PCR). The reaction was initiated by 5-min incubation at 94 ℃; 35 cycles of 94 ℃ for 45 s, 56 ℃ for 45 s, 72 ℃ for 60 s; and terminated after a 10-min extension at 72 ℃. Products were purified by gel extraction. Recombinant plasmids were transferred into *Escherichia coli* DH5α, and identified by restriction enzymes digestion and sequencing analysis. Then, we constructed MAWBP and MAWD short hairpin RNA (shRNA) plasmids. Oligonucleotides were annealed and ligated to pSilencer3.1-H1-Neo. All of the primers are shown on Table 1.

Real-time PCR

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, United States), and subjected (5 μg) to RT-PCR (Table 1). The internal control, β-actin, was processed with all specimens simultaneously. Real-time PCR was performed using Applied Biosystem 7500 Real-Time PCR System (Foster City, CA, United States). Data were analyzed using the relative standard curve method.

Western blotting

Proteins were extracted from cells for western blotting. Proteins (50 μg) were separated on sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinyl difluoride membranes (Bio-Rad, Hercules, CA, United States). Immunoreactivity was tested with anti-MAWD (1:500, our laboratory), anti-MAWBP (1:500, our laboratory)[11], E-cadherin (1:500, BD, Franklin Lakes, NJ, United States), N-cadherin (1:500, BD), Snail (1:500, Cell Signaling, Danvers, MA, United States), diluted in blocking buffer at 4 ℃ overnight. The signal was detected by Super Signal West Dura Extended Duration Substrate (Thermo Scientific, Rockford, IL, United States).

Transfection studies

SGC7901 cells were transfected with overexpression plasmids, while BGC823 cells were transfected with shRNA plasmids. Cells were cultured at 60%-70% confluence in 35-mm plates and were transfected using Lipofectamine 2000 (Invitrogen). Except that mono-plasmids and empty vector were transfected into GC cells, overexpressed plasmids of MAWBP and MAWD were cotransfected into SGC7901 cells. shRNA plasmids of MAWBP and MAWD were cotransfected into BGC823 cells. At 48 h post-transfection, cells were seeded for 21 d in selection medium containing 400 μg/mL G418, to screen for stable clones. The efficacy of transfection was identified by RT-PCR and Western blotting.

Table 1 List of oligonucleotide primers

Target gene	Primer ID	Sequence (5'-3')
MAWBP (132 bp)	Forward	GGGTCTGCACACGCTGTTC
	Reverse	TAATGTCAACCCTTCCGTCT
MAWD (162 bp)	Forward	GGGACAGGATAAACTTTAGC
	Reverse	AGCATGATCCCAAAGTCGAAC
MAWBP (867 bp)	Forward	AACTTGGTCGACCAGCTTGCAAGG AAAATG
	Reverse	ATAACTCGAGCTAGGCTGTCAGTGT GCC
MAWD (1053 bp)	Forward	CGCGGATCCATGGCAATGAGACAG ACG
	Reverse	CCCAAGCTTTCAGGCCTTAACATCA GG
β-actin (510 bp)	Forward	CGGGAAATCGTGCGTGACATT
	Reverse	CTAGAAGCATTTGCGGTGGAC
β-actin (150 bp)	Forward	TTAGTTGCGTTACACCCTTTC
	Reverse	ACCTTCACCGTTCCAGTTT
MAWD (shRNA)	Ps-F1	GATCCGCTTATGGACGATCTATTGC TTCAAGAGAGCAATAGATCGTCCAT AAGTTTTTTGGAAA
	Ps-R1	AGCTTTTCCAAAAAACTTATGGACG ATCTATTGCTCTCTTGAAGCAATAG ATCGTCCATAAGCG
MAWBP (shRNA)	Ps-F1	GATCCGTAGCACGCTCACGTTTGTC TTCAAGAGAGACAAACGTGAGCGT GCTATTTTTTGGAAAAGC
	Ps-R1	AGCTTGGCGTAATCATGGTCATAGC TGTTTCCTGTGTGAAATTGTTATCCG CTCACAATTCCACACA

MAWD: Mitogen-activated protein kinase activator with WD40 repeats; MAWBP: MAWD binding protein; shRNA: Short hairpin RNA.

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide assay

Stable transfected cells (1×10^3) in 200 μL DMEM supplemented with 5% FBS were seeded in duplicate into each well of 96-well culture plates, and 10 μL 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT, GenView, Jacksonville, FL, United States) (5 mg/mL) was added at 0, 24, 48, 72 and 96 h. The MTT was removed after 4 h incubation, and 100 μL dimethylsulfoxide (Amresco, Solon, OH, United States) was pipetted into each well and incubated for 30 min. Absorbance was measured at 570 nm using an iMark Microplate Reader (Bio-Rad, Hercules, CA, United States).

Soft agar colony formation assay

Cells (3×10^3) were trypsinized and resuspended in 4 mL 0.3% agar in DMEM containing 10% FBS, and overlaid with 0.6% agar in 60-mm culture dishes. The dishes were incubated routinely for 21 d. Colonies were stained with 0.2% p-iodonitrotetrazolium violet, photographed,

and counted.

Tumorigenicity assay in nude mice

Animal experiments were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Ethics Committee of Peking University. All efforts were made to minimize suffering. Transfected cells were resuspended in 1× Hank's Buffer at a concentration of 5×10^5 cells/mL. A 100-μL suspension was injected subcutaneously into the left dorsal flank of 15 5-wk-old female nude mice, and the right side was inoculated with GC cells transfected by vector alone as a control. The mice were checked every 3 d for tumor appearance, and the large (a) and small (b) diameters of the palpable tumors were recorded for tumor volume calculation according to $a \times b^2 \times 0.5$.

Wound healing assay

Cells were cultured at 80%-90% confluence in 60-mm dishes. Cells were scratched with a pipette tip to produce a straight line. The detached cells were washed three times with phosphate buffered solution (PBS) and incubated for a further 24 h. The scratched gap was inspected at 0, 2, 4, 6, 12 and 24 h. Photographs were taken at × 200 magnification, using a TS100 inverted microscope (Nikon, Tokyo, Japan).

Transwell assay

The invasion assay was performed using a BD Matrigel Invasion Chamber. Cells (1×10^5) were suspended in serum-free DMEM and seeded on matrix membranes. DMEM supplemented with 10% FBS was used as a chemoattractant. After 48 h incubation, cells were fixed with methanol and stained with crystal violet for 20 min. Cells that penetrated the membrane were counted.

Immunofluorescence

Cells were grown on glass slides, washed with PBS, fixed in methanol for 10 min, and processed for immunofluorescence. Cells were exposed to anti-p-Smad3 overnight at 4 ℃, incubated for 1 h with rhodamine-conjugated anti-rabbit secondary antibodies, and nuclei were stained with 4',6-diamidino-2-phenylindole. Cells were studied with a confocal fluorescence imaging microscope (TCS-SP5; Leica, Mannheim, Germany).

Analysis of TGF-β pathway responses

Cells were starved for 24 h, and then incubated in 5% FBS–DMEM containing 2 or 4 ng/mL TGF-β for 24 or 48 h. Plasminogen activator inhibitor (PAI)-1 promoter assays were used to select the optimum TGF-β conditions. Transfected cells were cultured in 5% FBS-DMEM containing 4 ng/mL TGF-β for 24 h. PhosphoSafe Extraction Reagent (Merck, San Diego, CA, United States) was used to extract phosphoprotein. P-Smad3 (1:1000, Abcam, Cambridge, United Kingdom) and p-Smad2 (1:500, Millipore, Temecula, CA, United States) were analyzed by western blotting as described above. Smad2 (1:500, Bioworld, Boston, MA, United States) and Smad3 (1:500, Bioworld) were detected at the same time. We then separated the cytosolic and nuclear fractions according to the protocol of the Nuclear-Cytosol Extraction Kit (Applygen Technologies Inc., Beijing, China) and detected p-Smad3 levels. Nuclear translocation ability of p-Smad3 (1:50) was analyzed by confocal microscopy as described above.

Ethics

The study has been examined and approved ethically by the Ethics Committee of Beijing Cancer Hospital.

Statistical analysis

Statistical analysis used SPSS version 16.0. Student's *t* test and analysis of variance were used for data measurement. Quantitative values were presented as mean ± SD. Differences with $P < 0.05$ were considered statistically significant.

RESULTS

MAWBP and MAWD inhibited proliferation and tumorigenicity of GC cells

BGC823, MGC803, SGC7901, AGS, N87 and MKN45 GC cell lines were used to detect MAWBP and MAWD expression, which was found to differ between the cell types. RT-PCR and real-time PCR revealed low levels of endogenous MAWBP and MAWD mRNA in SGC7901 cells but high levels in BGC823 cells, and Western blotting confirmed these results (Figure 1). These two cell lines were selected for the following experiments.

To investigate further biological function of MAWBP and MAWD in GC cells, MAWBP-pcDNA3.1 and MAWD-pcDNA3.1, alone or in combination, and empty vector were transfected into SGC7901 cells, in which these two proteins were expressed at a lower level compared with other GC cells. G418-resistant clones were isolated, which were stably transfected cells. These cells were termed as MAWBP, MAWD, MAWBP/D (MAWBP and MAWD cotransfected cells) and vector, respectively. shRNA plasmid MAWBP-pSilencer3.1 and MAWD-pSilencer3.1, alone or in combination, and empty vector were transfected into BGC823 cells, in which they were expressed at a higher level compared with other GC cells. The stably transfected cells were termed as sh-MAWBP, sh-MAWD, sh-MAWBP/D (MAWBP and MAWD co-downregulated groups) and sh-vector, respectively. We analyzed MAWBP and MAWD expression at the mRNA and protein levels by semi-quantitative RT-PCR and Western blotting, respectively.

In the MTT assay, cell growth in the overexpressed group was suppressed in the MAWBP, MAWD and MAWBP/D groups, compared with the vector group (Figure 2A, $P < 0.001$). Knockdown of MAWBP and MAWD in BGC823 cells using RNA interference (RNAi) increased cell growth (Figure 2B, $P < 0.001$). The soft agar assay in overexpressed cells showed reduced colony formation for the MAWBP, MAWD, and MAWBP/D

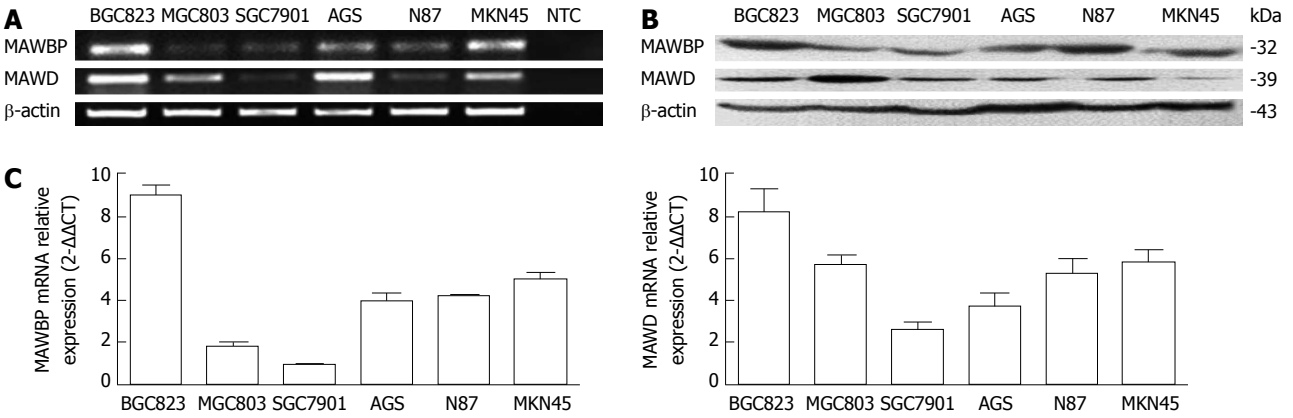


Figure 1 Analysis of expression of mitogen-activated protein kinase activator with WD40 repeats binding protein and mitogen-activated protein kinase activator with WD40 repeats in six gastric cancer cell lines. A: mRNA expression for mitogen-activated protein kinase activator with WD40 repeats (MAWD)/MAWD binding protein (MAWBP) in BGC823, MGC803, SGC7901, AGS, N87 and MKN45 six gastric cancer cell lines was detected by reverse transcription-polymerase chain reaction (PCR). There was a low level of endogenous MAWBP and MAWD mRNA in SGC7901 cells, and a high level in BGC823 cells; B: Expression of MAWBP and MAWD proteins was detected by Western blotting. The expression level was lower in SGC7901 cells than in the other cell lines. Expression of MAWBP and MAWD was higher in BGC823 cells than in the other cells; C: mRNA expression for MAWBP and MAWD was detected by real-time PCR. There were lower levels of endogenous MAWD and MAWBP mRNA in SGC7901 cells, and higher levels in BGC823 cells. NTC: No template control.

groups for cell number and size compared with the vector group (Figure 2C, clones number: 86.25 ± 8.43, 12.75 ± 4.49, 30 ± 6.41 *vs* 336.75 ± 22.55, $P < 0.001$). The corresponding knockdown group demonstrated the opposite effects (Figure 2D, 131.25 ± 16.54, 88.75 ± 11.12, 341.75 ± 22.23 *vs* 30.25 ± 8.07, $P < 0.001$). These results suggested that expression of MAWBP and MAWD play a role in inhibiting proliferation of GC cells.

In vivo experiments, tumor growth appeared to be slow in nude mice injected with MAWBP, MAWD, and MAWBP/D compared with the control group. Tumors from MAWD-transfected cells were smaller than those from the other groups (Figure 2E and F, $P < 0.001$).

Co-expression of MAWBP and MAWD suppressed migration and invasion of GC cells

To explore the potential role of MAWBP and MAWD in GC metastasis and invasion, we evaluated the effects of the stably transfected cells on migration and invasion using a wound healing assay and transwell invasive activity assay, respectively. In the wound healing assay, the scratch gap of vector transfected cells was almost closed at 24 h in the overexpressed group. Cells with co-overexpression of MAWBP and MAWD showed the slowest rate of migration (Figure 3A). In the knockdown group, the cells with combined downregulation of MAWBP and MAWD expression migrated faster than the other cells. Migration of sh-vector cells was slowest (Figure 3B). In the transwell assay, there was significant difference in the number of the cells traversing the matrix membrane between the vector and other groups. Combined overexpression of MAWBP and MAWD decreased the invasive ability of GC cells (Figure 4A). The number of traverse cells of MAWBP, MAWD and co-expression group was higher than that in the vector group (Figure 4C, 84 ± 16.57, 98.33 ± 9.8, 29 ± 16.39 *vs* 298 ± 11.86, $P < 0.001$). Knockdown of expression of MAWBP and MAWD increased the number of cells that traversed the matrix membrane (Figure 4B). Cells with combined downregulation of MAWBP and MAWD expression migrated faster than the other cells (Figure 4B). Invasion of sh-vector cells was slowest (Figure 4C, 100.67 ± 14.57, 72.66 ± 8.51, 330.67 ± 20.55 *vs* 27 ± 11.53, $P < 0.001$). These data showed that MAWBP and MAWD inhibited migration and invasion of GC cells.

Co-expression of MAWBP and MAWD influenced expression of EMT markers induced by TGF-β1 in GC cells

EMT contributes to cancer progression and metastasis. TGF-β is the main and best-characterized inducer of EMT. We sought to determine whether co-expression of MAWBP and MAWD inhibited TGF-β1-induced EMT, thus suppressing migration and transwell behavior of GC cells. We detected a relationship between the expression of MAWBP and MAWD and the EMT markers induced by TGF-β1, such as E-cadherin, N-cadherin, and transcription factor Snail. We established the optimum TGF-β1 concentration and treatment time to stimulate cells. We used the expression of TGF-β reporter gene PAI-1 to indicate that the optimum TGF-β1 concentration and treatment time was 4 ng/mL for 24 h (Figure 5A). We stimulated GC cells that overexpressed MAWBP and MAWD with TGF-β1, and observed their morphology. We found that cells that overexpressed both MAWBP and MAWD displayed a pebble-like shape and tight cell-cell adhesion, while vector-treated cells showed a classical mesenchymal phenotype (Figure 5B). That means that co-expression of MAWBP and MAWD inhibited morphological changes of TGF-β1-induced EMT. We next detected expression of E-cadherin, N-cadherin and Snail in cells overexpressing MAWBP and MAWD. Ex-

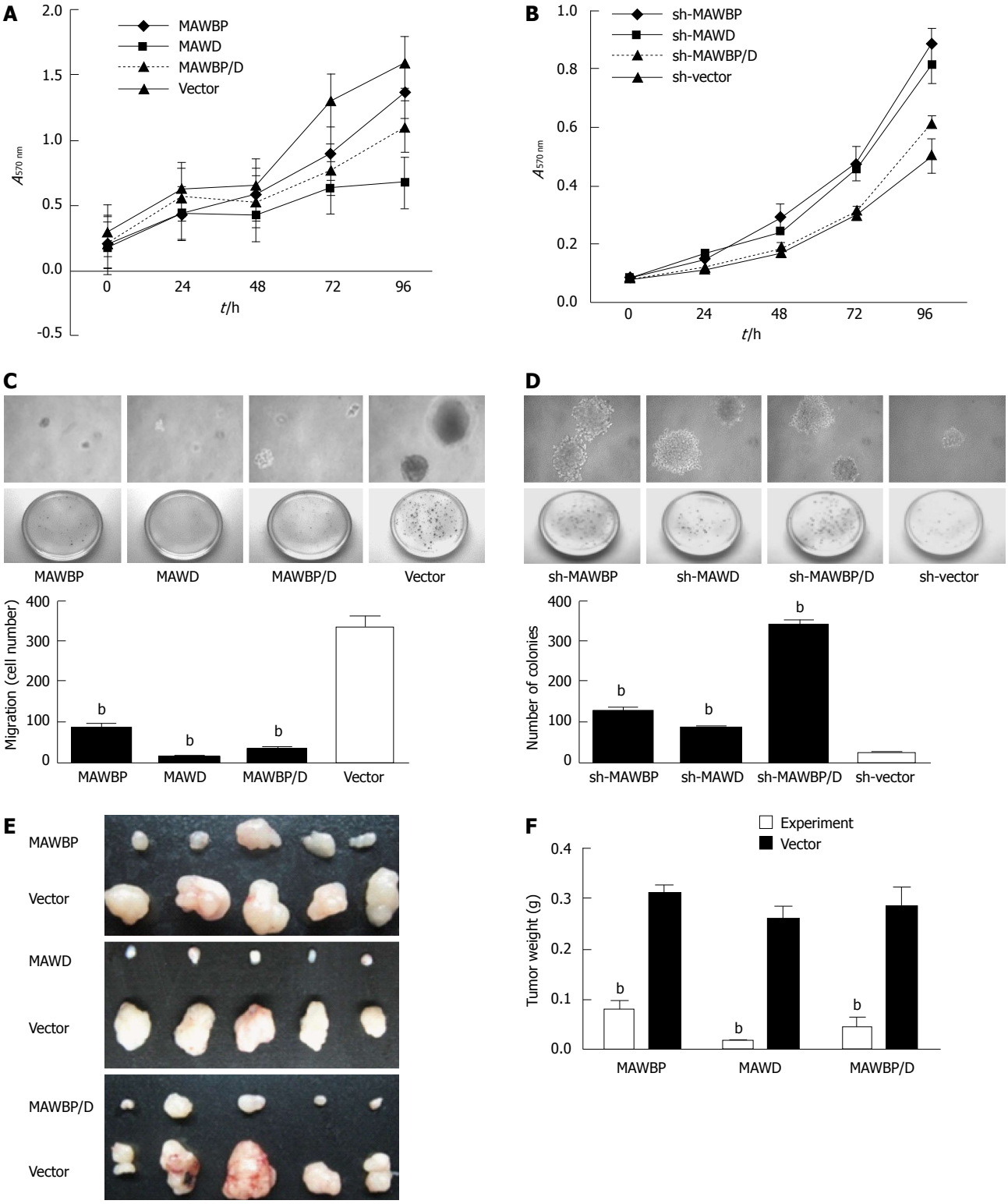
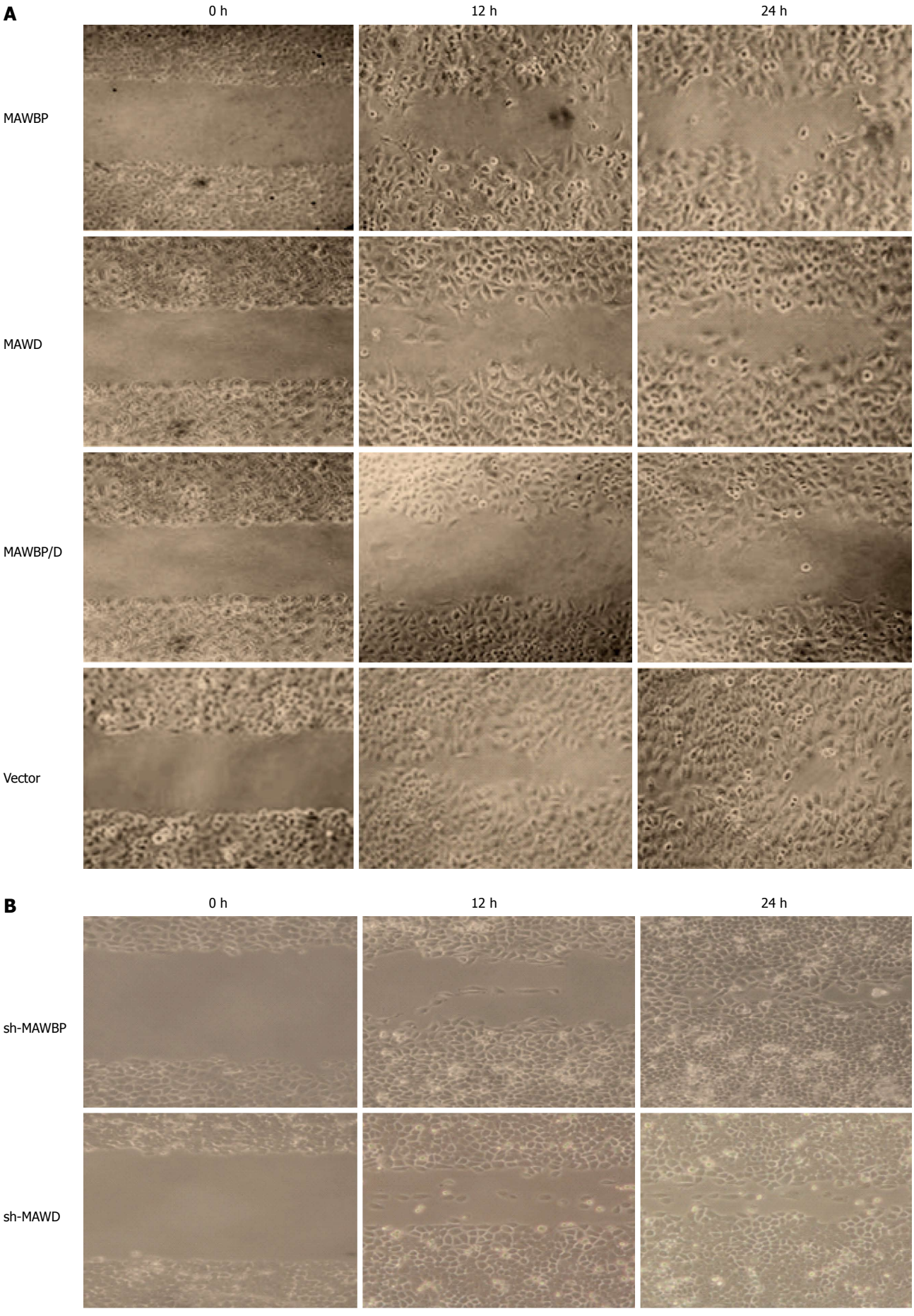


Figure 2 Effect of mitogen-activated protein kinase activator with WD40 repeats binding protein and mitogen-activated protein kinase activator with WD40 repeats on proliferation and tumorigenicity of gastric cancer cells. A: 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide assay showed that growth of SGC7901 cells overexpressing mitogen-activated protein kinase activator with WD40 repeats (MAWD)/MAWD binding protein (MAWBP) was markedly inhibited; B: In BGC823 cells with inhibition of expression of MAWBP and MAWD, cell growth was inversely increased; C: In soft agar assay, colonies of cells with overexpression of MAWBP, MAWD and MAWBP/D were reduced in number and size compared with cells transfected with vectors alone (original magnification of clones: ×100); D: Knockdown group demonstrated the opposite effects. There was an increase in the number and size of sh-MAWBP, sh-MAWD, sh-MAWBP/D cells compared with cells transfected with vectors alone; E: Nude mouse xenografts. Tumors induced by MAWBP, MAWD and MAWBP/D cotransfected cells were smaller than those of the vector group; F: Xenograft weight. The average tumor weight were calculated from five nude mice in every group. Data are presented as the mean ± SD from three independent experiments. $^{b}P < 0.01$ *vs* vector group.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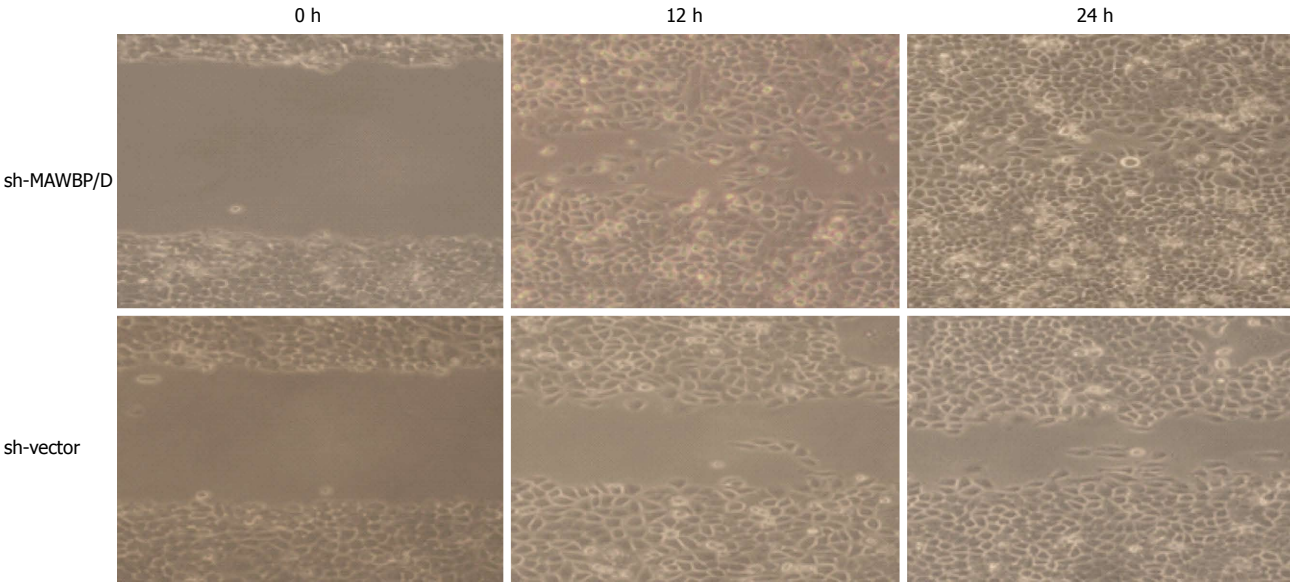

Figure 3 Effect of mitogen-activated protein kinase activator with WD40 repeats binding protein and mitogen-activated protein kinase activator with WD40 repeats on migration of gastric cancer cells. A: Wound healing assay showed that migration of vector-transfected cells was faster than that of cells overexpressing mitogen-activated protein kinase activator with WD40 repeats (MAWD)/MAWD binding protein (MAWBP). The scratch gap in vector group was almost closed at 24 h. The migration of MAWBP/D cotransfected cells was slowest; B: Cells with combined downregulation of MAWBP and MAWD expression migrated faster than the other cells. Migration of sh-vector cells was slowest (original magnification, ×100).

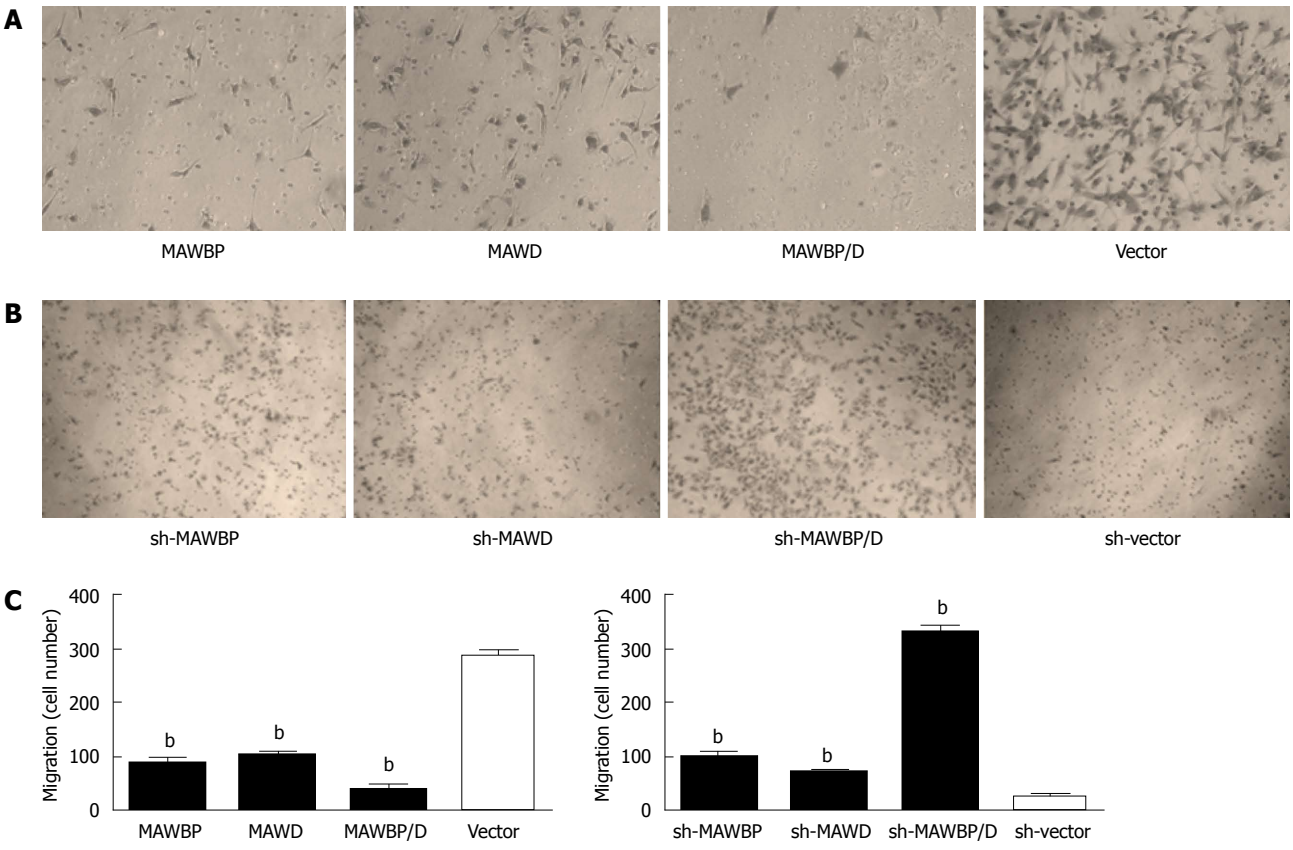

Figure 4 Effect of mitogen-activated protein kinase activator with WD40 repeats binding protein and mitogen-activated protein kinase activator with WD40 repeats on invasive ability of gastric cancer cells. A: Transwell assay showed that invasive ability of mitogen-activated protein kinase activator (MAWD) with WD40 repeats binding protein (MAWBP)/D cotransfected cells was weakest, with the lowest number of cells to cross the matrix membranes. Vector-transfected cells migrated farthest; B: Knockdown of MAWBP and MAWD increased the invasive ability of gastric cancer (GC) cells (original magnification: ×100); C: Number of cells that traverse the matrix membrane in the different groups. Data are presented as the mean ± SD from three independent experiments. $^{b}P < 0.01$ *vs* vector group.

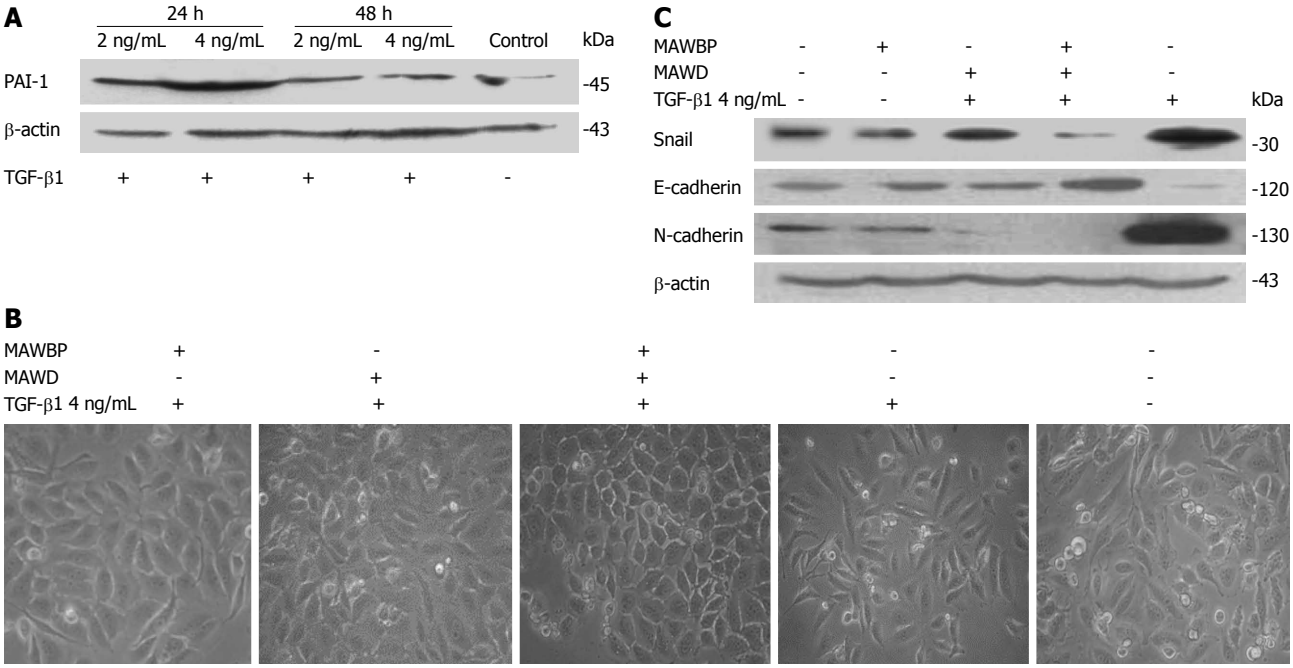

Figure 5 Effect of mitogen-activated protein kinase activator with WD40 repeats binding protein and mitogen-activated protein kinase activator with WD40 repeats on expression of biomarkers specific for epithelial-mesenchymal transition induced by transforming growth factor-β1. A: According to the expression of transforming growth factor (TGF)-β downstream reporter gene plasminogen activator inhibitor (PAI)-1, the optimum TGF-β1 concentration and time were confirmed as 4 ng/mL for 24 h; B: SGC7901 cells overexpressing mitogen-activated protein kinase activator with WD40 repeats (MAWD)/MAWD binding protein (MAWBP) were stimulated by 4 ng/mL TGF-β1 for 24 h. Cells that overexpressed both MAWBP and MAWD displayed a pebble-like shape, while vector cells showed a classical mesenchymal phenotype (original magnification, ×400); C: Expression of E-cadherin was strongest in the MAWBP/D cotransfection group and weakest in the vector group, using Western blotting. Snail and N-cadherin were inversely associated with E-cadherin expression.

pression of E-cadherin was strongest in the MAWBP/D group and weakest in the vector group. N-cadherin and Snail expression was inversely associated with E-cadherin expression (Figure 5C). Collectively, these data demonstrated that MAWBP and MAWD were involved in TGF-β1-induced EMT through upregulating E-cadherin and downregulating N-cadherin and Snail in GC cells.

Co-expression of MAWBP and MAWD suppressed phosphorylation and nuclear translocation of p-Smad3

Following MAWBP and MAWD overexpression in cells stimulated with TGF-β1, the level of p-Smad3 was lowest in the MAWBP/D group and highest in the vector group (Figure 6A). The level of p-Smad2 was also lower in the MAWBP/D group. Furthermore, we separated the proteins in the cytoplasm and nucleus and found that p-Smad3 in the nucleus had the lowest level, as shown by Western blotting (Figure 6B) and confocal microscopy (Figure 6C). That means that the nuclear translocation capability of p-Smad3 in cotransfected cells was weakest. These results imply that the MAWBP/D complex suppressed TGF-β signaling by inhibiting downstream phosphorylation.

DISCUSSION

From the outset of this study, we analyzed the biological function of MAWBP and MAWD in GC cell lines. We found that MAWBP and MAWD inhibited proliferation and migration of GC cells. Importantly, combined overexpression of MAWBP and MAWD in GC cells suppressed TGF-β1-induced EMT by attenuating phosphorylation of Smad3 and reducing its nuclear translocation.

In a previous study, we reported proteomic data acquired from screening protein profiles from GC tissues, including MAWBP and MAWD, and showed that they formed a complex in GC cells by co-immunoprecipitation[11]. MAWD has been reported to have divergent effects in cancer. Some researchers have suggested that MAWD promotes cancer development. Matsuda *et al*[12] found that MAWD was overexpressed in 45.6% (21/46) of human breast tumor tissues, and promoted anchorage-independent cell growth. Kim *et al*[15] reported MAWD upregulation in 50.8% (30/59) of adenomas and 70.7% (87/123) of colorectal cancers. Halder *et al*[23] found that STRAP was upregulated in 60% (12/20) of colon and 78% (11/14) of lung carcinomas. However, other researchers have found that MAWD suppressed development of malignant cells. Buess *et al*[24] reported complete or partial allelic loss of MAWD in 45.2% (75/166) of colorectal cancer patients. Jung *et al*[25] found that MAWD was a binding partner of NM23-H1, creating a complex that interacted with and potentiated p53. Dong *et al*[26] detected chromosomal deletions in prostate cancer that overlapped *MAWD* gene locations. Zhao *et al*[27] reported that MAWBP was downregulated in ulcerative colitis. The function of MAWBP and MAWD in GC has not been reported.

In the present study, we investigated the biological

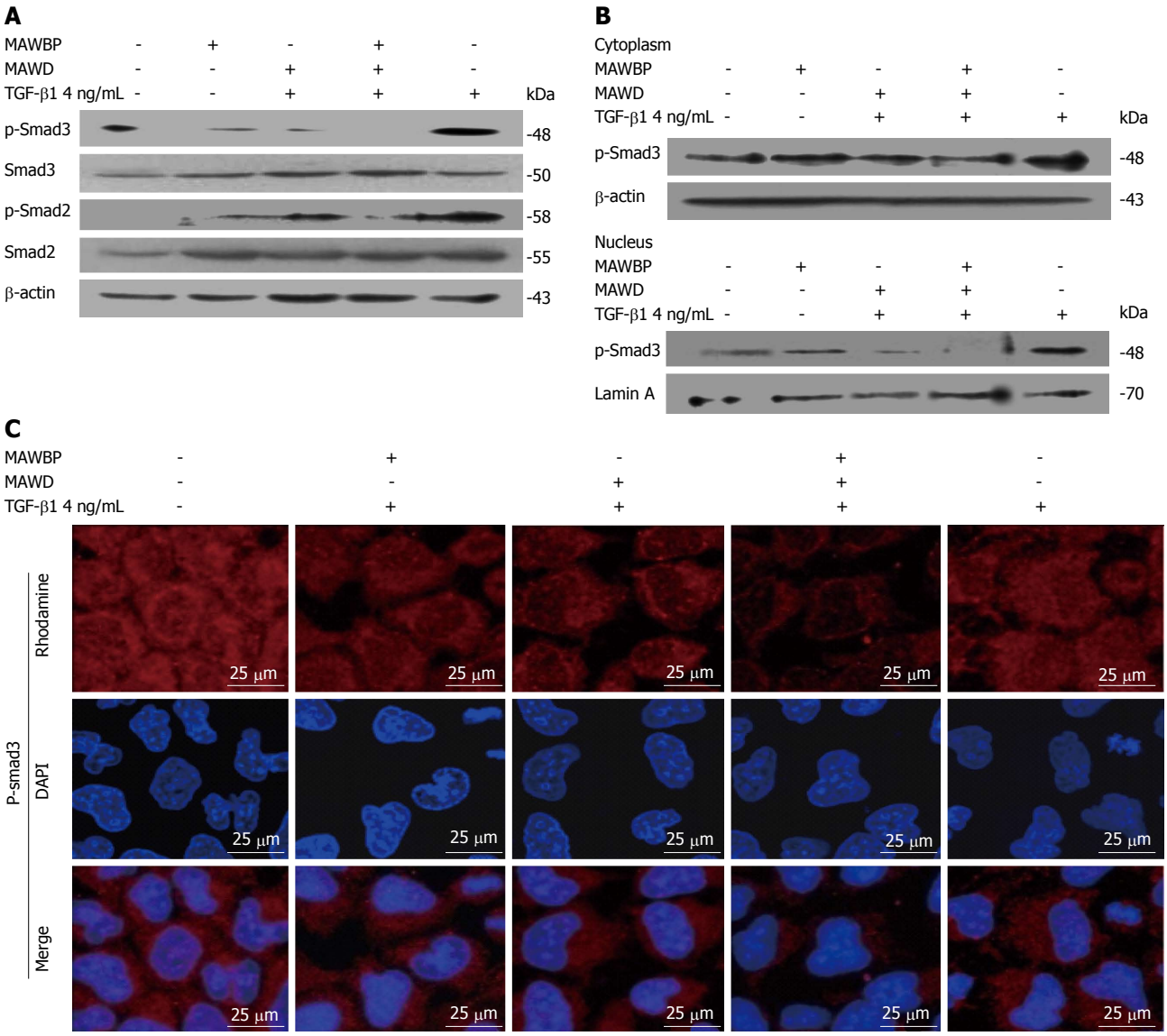

Figure 6 Combination of mitogen-activated protein kinase activator with WD40 repeats binding protein and mitogen-activated protein kinase activator with WD40 repeats inhibited the transforming growth factor-β pathway. A: The level of p-Smad3 was lowest in the mitogen-activated protein kinase activator (MAWD) with WD40 repeats binding protein (MAWBP)/D cotransfected group and highest in the vector group. p-Smad2 was also lower in the MAWBP/D group; B, C: Nuclear translocation capability of p-Smad3 in cotransfected cells was weakest that means the activity of transforming growth factor (TGF)-β pathway in co-expression group was inhibited. DAPI: 4',6-diamidino-2-phenylindole.

role of MAWBP and MAWD in GC. We first investigated expression levels of MAWBP and MAWD in six GC cell lines at the RNA and protein levels. We found that expression was lowest in SGC7901 cells and highest in BGC823 cells. Thus, we selected SGC7901 for overexpression of MAWBP and MAWD and BGC823 for RNAi assay, and constructed the eukaryotic expression plasmid and RNAi plasmid for these two proteins for cell transfection. We generated MAWBP/D-cotransfected cells to establish whether one complements the function of the other. We found that MAWBP and MAWD acted as tumor suppressors. Our results showed that overexpression of MAWBP and MAWD suppressed growth of SGC7901 cells. Knockdown of their expression enhanced proliferation of BGC823 cells. The suppressive ability of MAWD was more pronounced than that of MAWBP. Interestingly, the results from the migration and transwell assays indicated that combined overexpression of these two proteins more obviously limited migration and invasive behavior of GC cells. The cotransfected cells showed mixed characteristics for proliferation and migration, meaning that MAWBP and MAWD had a synergetic role in regulating migration and invasion of GC cells.

EMT is thought to be a key step in the progression of tumors toward invasion and metastasis[28]. EMT is a cellular process during which epithelial polarized cells become motile mesenchymal-appearing cells. This process can lead to loss of epithelial markers - especially E-cadherin - and expression of mesenchymal markers such as vimentin and N-cadherin[29]. E-cadherin is a cell-adhesion protein that is regulated by transcription factors including Snail and Slug. Snail act as a repressor and blocks E-cad-

herin transcription, and has emerged as an essential regulator of physiological and pathological EMT processes[30]. It has been shown that TGF-β induces changes in cell morphology that are consistent with the acquisition of the EMT phenotype[31].

In this study, we sought to determine whether co-expression of MAWBP and MAWD inhibited TGF-β1-induced EMT, thus suppressing migration and transwell behavior of GC cells. We stimulated GC cells with overexpression of MAWBP and MAWD with TGF-β1 and detected the expression level of epithelial and mesenchymal markers and transcription factors. We found that E-cadherin was upregulated in the co-expression group and N-cadherin and Snail expression was inversely associated with E-cadherin expression. This revealed that MAWBP and MAWD had a synergetic function in inhibiting TGF-β1-induced EMT.

TGF-β1 stimulation induces upregulation of Snail and induces EMT in Smad-dependent signaling[30]. MAWD was found to recruit Smad7 and form a complex that inhibited TGF-β signaling. To confirm whether the MAWBP and MAWD complex further suppressed TGF-β1 and decreased Snail expression, we evaluated TGF-β activity in MAWBP and MAWD overexpressed cells. Phosphorylation of effector molecules is often essential for downstream receptor kinase signaling[31]. Thus, we detected the phosphorylation level and nuclear translocation of p-Smad2 and p-Smad3 to indicate TGF-β activity. We found that the level of p-Smad3 was lowest in the combined overexpression group and highest in the vector group. Nuclear translocation of p-Smad3 was weakest in cells with combined overexpression. These results imply that the MAWBP/D complex suppresses TGF-β signaling, and therefore downregulates Snail level and inhibits EMT.

All together, the present study demonstrates that MAWBP and MAWD have a suppressive role in progression of tumor growth and invasion of GC. Co-expression of MAWBP and MAWD inhibits TGF-β1-induced EMT, which suppresses EMT-assisted GC cell malignant progression. In future research, we should attempt to find the mechanisms mediating MAWBP and MAWD expression in GC. MAWBP and MAWD interaction domains will be predicted by biological information analysis and tested in cell assays.

ACKNOWLEDGMENTS

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COMMENTS

Background

Gastric cancer (GC) is the second most common cause of cancer death worldwide. GC incidence in Asian countries, particularly in East Asia, is significantly higher than that in other parts of the world. GC creates a serious public health problem. Early diagnosis is important for therapy and prognosis of patients. Therefore, investigation of sensitive biomarkers and analysis of their function are important.

Research frontiers

During the past decade, a great effort has been made to define better the biological profile of GC. Molecular genetic studies have investigated the accumulation of mutations and alterations in proteins involved in GC. These include activation of c-myc, erbB-2, c-met, and k-ras oncogenes and inactivation of tumor suppressor genes *p53*, *APC*, *E-cadherin* and *RUNX3*. Their previous study found that mitogen-activated protein kinase activator with WD40 repeats (MAWD) and MAWD binding protein (MAWBP) were differentially expressed and interacted in GC.

Innovations and breakthroughs

The present study provided direct evidence that MAWBP and MAWD inhibited proliferation and migration of GC cells. Importantly, interaction of MAWBP and MAWD influenced expression of epithelial-mesenchymal transition (EMT) markers induced by transforming growth factor (TGF)-β1 in GC cells. It also reduced nuclear translocation of p-Smad3. This means that co-expression of MAWBP and MAWD inhibits TGF-β1-induced EMT and suppresses EMT-aided GC malignant progression.

Applications

The authors found that interaction between MAWBP and MAWD could shed new light on the carcinogenic mechanisms of GC. MAWBP and MAWD as biomarkers might be diagnostic and therapeutic targets for GC.

Terminology

MAWD is a protein that is evolutionarily conserved and is widely expressed in many tissues. Sequence analysis indicates that the protein structure of MAWD contains a WD40 repeat domain. WD repeat proteins help to assemble macromolecular complexes, such as shown for the β-subunit of G proteins. The homologous protein of MAWD, serine-threonine kinase receptor-associated protein recruits Smad7 to the activated type I receptor and forms a complex. MAWBP is a MAWD binding protein. EMT is the morphological and molecular changes that occur when epithelial cells lose their characteristics, gain mesenchymal properties and become motile, which is a key event in tumor invasion and metastasis.

Peer review

The authors analyzed MAWD and MAWBP in a series of GC cell lines. They found that co-expression of both genes is potentially involved in the suppression of migration and invasion in their selected cell lines. This study was a straightforward continuation of the authors' own work and they have recently reported differential expression of both genes in GC tissues. Now, they have analyzed the functional consequences of suppression or overexpression of both genes in an *in vitro* setting.

REFERENCES

1 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078]
2 **Becker K**, Langer R, Reim D, Novotny A, Meyer zum Buschenfelde C, Engel J, Friess H, Hofler H. Significance of histopathological tumor regression after neoadjuvant chemotherapy in gastric adenocarcinomas: a summary of 480 cases. *Ann Surg* 2011; **253**: 934-939 [PMID: 21490451 DOI: 10.1097/SLA.0b013e318216f449]
3 **Hass HG**, Smith U, Jäger C, Schäffer M, Wellhäuber U, Hehr T, Markmann HU, Nehls O, Denzlinger C. Signet ring cell carcinoma of the stomach is significantly associated with poor prognosis and diffuse gastric cancer (Lauren's): single-center experience of 160 cases. *Onkologie* 2011; **34**: 682-686 [PMID: 22156447 DOI: 10.1159/000334545]
4 **Leal MF**, Calcagno DQ, Borges da Costa Jde F, Silva TC, Khayat AS, Chen ES, Assumpção PP, de Arruda Cardoso Smith M, Burbano RR. MYC, TP53, and chromosome 17 copy-number alterations in multiple gastric cancer cell lines and in their parental primary tumors. *J Biomed Biotechnol* 2011; **2011**: 631268 [PMID: 21528007 DOI: 10.1155/2011/631268]
5 **Cidon EU**, Centeno RG, Lagarto EG, Peral JI. HER-2 Evaluation in a Specific Gastric Cancer Population with the Highest Rate of Mortality in Spain. *J Oncol* 2011; **2011**: 391564

[PMID: 22131990 DOI: 10.1155/2011/391564]
6 **Zhao J**, Zhang X, Xin Y. Up-regulated expression of Ezrin and c-Met proteins are related to the metastasis and prognosis of gastric carcinomas. *Histol Histopathol* 2011; **26**: 1111-1120 [PMID: 21751142]
7 **Matkar SS**, Durham A, Brice A, Wang TC, Rustgi AK, Hua X. Systemic activation of K-ras rapidly induces gastric hyperplasia and metaplasia in mice. *Am J Cancer Res* 2011; **1**: 432-445 [PMID: 21761008]
8 **Mimata A**, Fukamachi H, Eishi Y, Yuasa Y. Loss of E-cadherin in mouse gastric epithelial cells induces signet ring-like cells, a possible precursor lesion of diffuse gastric cancer. *Cancer Sci* 2011; **102**: 942-950 [PMID: 21276134 DOI: 10.1111/j.1349-7006.2011.01890.x]
9 **Li QL**, Ito K, Sakakura C, Fukamachi H, Inoue Ki, Chi XZ, Lee KY, Nomura S, Lee CW, Han SB, Kim HM, Kim WJ, Yamamoto H, Yamashita N, Yano T, Ikeda T, Itohara S, Inazawa J, Abe T, Hagiwara A, Yamagishi H, Ooe A, Kaneda A, Sugimura T, Ushijima T, Bae SC, Ito Y. Causal relationship between the loss of RUNX3 expression and gastric cancer. *Cell* 2002; **109**: 113-124 [PMID: 11955451]
10 **Lu M**, Zhang L, Maul RS, Sartippour MR, Norris A, Whitelegge J, Rao JY, Brooks MN. The novel gene EG-1 stimulates cellular proliferation. *Cancer Res* 2005; **65**: 6159-6166 [PMID: 16024617 DOI: 10.1158/0008-5472.CAN-04-4016]
11 **Zhang J**, Kang B, Tan X, Bai Z, Liang Y, Xing R, Shao J, Xu N, Wang R, Liu S, Lu Y. Comparative analysis of the protein profiles from primary gastric tumors and their adjacent regions: MAWBP could be a new protein candidate involved in gastric cancer. *J Proteome Res* 2007; **6**: 4423-4432 [PMID: 17929853 DOI: 10.1021/pr0703425]
12 **Matsuda S**, Katsumata R, Okuda T, Yamamoto T, Miyazaki K, Senga T, Machida K, Thant AA, Nakatsugawa S, Hamaguchi M. Molecular cloning and characterization of human MAWD, a novel protein containing WD-40 repeats frequently overexpressed in breast cancer. *Cancer Res* 2000; **60**: 13-17 [PMID: 10646843]
13 **Iriyama C**, Matsuda S, Katsumata R, Hamaguchi M. Cloning and sequencing of a novel human gene which encodes a putative hydroxylase. *J Hum Genet* 2001; **46**: 289-292 [PMID: 11355021 DOI: 10.1007/s100380170081]
14 **Datta PK**, Moses HL. STRAP and Smad7 synergize in the inhibition of transforming growth factor beta signaling. *Mol Cell Biol* 2000; **20**: 3157-3167 [PMID: 10757800]
15 **Kim CJ**, Choi BJ, Song JH, Park YK, Cho YG, Nam SW, Yoo NJ, Lee JY, Park WS. Overexpression of serine-threonine receptor kinase-associated protein in colorectal cancers. *Pathol Int* 2007; **57**: 178-182 [PMID: 17316412 DOI: 10.1111/j.1440-1827.2007.02078.x]
16 **Suh Y**, Yoon CH, Kim RK, Lim EJ, Oh YS, Hwang SG, An S, Yoon G, Gye MC, Yi JM, Kim MJ, Lee SJ. Claudin-1 induces epithelial-mesenchymal transition through activation of the c-Abl-ERK signaling pathway in human liver cells. *Oncogene* 2012 Nov 19; Epub ahead of print [PMID: 23160379 DOI: 10.1038/onc.2012.505]
17 **Chang CJ**, Chao CH, Xia W, Yang JY, Xiong Y, Li CW, Yu WH, Rehman SK, Hsu JL, Lee HH, Liu M, Chen CT, Yu D, Hung MC. p53 regulates epithelial-mesenchymal transition and stem cell properties through modulating miRNAs. *Nat Cell Biol* 2011; **13**: 317-323 [PMID: 21336307 DOI: 10.1038/ncb2173]
18 **Katsuno Y**, Lamouille S, Derynck R. TGF-β signaling and epithelial-mesenchymal transition in cancer progression. *Curr Opin Oncol* 2013; **25**: 76-84 [PMID: 23197193 DOI: 10.1097/CCO.0b013e32835b6371]
19 **Tran MN**, Choi W, Wszolek MF, Navai N, Lee IL, Nitti G, Wen S, Flores ER, Siefker-Radtke A, Czerniak B, Dinney C, Barton M, McConkey DJ. The p63 protein isoform ΔNp63α inhibits epithelial-mesenchymal transition in human bladder cancer cells: role of MIR-205. *J Biol Chem* 2013; **288**: 3275-3288 [PMID: 23239884 DOI: 10.1074/jbc.M112.408104]
20 **Merikallio H**, Turpeenniemi-Hujanen T, Pääkkö P, Mäkitaro R, Riitta K, Salo S, Salo T, Harju T, Soini Y. Snail promotes an invasive phenotype in lung carcinoma. *Respir Res* 2012; **13**: 104 [PMID: 23157169 DOI: 10.1186/1465-9921-13-104]
21 **Deng GR**, Lu YY, Chen SM, Miao J, Lu GR, Li H, Cai H, Xu XL, E Z, Liu PN. Activated c-Ha-ras oncogene with a guanine to thymine transversion at the twelfth codon in a human stomach cancer cell line. *Cancer Res* 1987; **47**: 3195-3198 [PMID: 3034404]
22 **Li Y**, Lu YY. Isolation of diallyl trisulfide inducible differentially expressed genes in human gastric cancer cells by modified cDNA representational difference analysis. *DNA Cell Biol* 2002; **21**: 771-780 [PMID: 12489988 DOI: 10.1089/104454902320908423]
23 **Halder SK**, Anumanthan G, Maddula R, Mann J, Chytil A, Gonzalez AL, Washington MK, Moses HL, Beauchamp RD, Datta PK. Oncogenic function of a novel WD-domain protein, STRAP, in human carcinogenesis. *Cancer Res* 2006; **66**: 6156-6166 [PMID: 16778189 DOI: 10.1158/0008-5472.CAN-05-3261]
24 **Buess M**, Terracciano L, Reuter J, Ballabeni P, Boulay JL, Laffer U, Metzger U, Herrmann R, Rochlitz C. STRAP is a strong predictive marker of adjuvant chemotherapy benefit in colorectal cancer. *Neoplasia* 2004; **6**: 813-820 [PMID: 15720808 DOI: 10.1593/neo.04307]
25 **Jung H**, Seong HA, Ha H. NM23-H1 tumor suppressor and its interacting partner STRAP activate p53 function. *J Biol Chem* 2007; **282**: 35293-35307 [PMID: 17916563 DOI: 10.1074/jbc.M705181200]
26 **Dong JT**. Chromosomal deletions and tumor suppressor genes in prostate cancer. *Cancer Metastasis Rev* 2001; **20**: 173-193 [PMID: 12085961]
27 **Zhao X**, Kang B, Lu C, Liu S, Wang H, Yang X, Chen Y, Jiang B, Zhang J, Lu Y, Zhi F. Evaluation of p38 MAPK pathway as a molecular signature in ulcerative colitis. *J Proteome Res* 2011; **10**: 2216-2225 [PMID: 21428429 DOI: 10.1021/pr100969w]
28 **Sehrawat A**, Kim SH, Vogt A, Singh SV. Suppression of FOXQ1 in benzyl isothiocyanate-mediated inhibition of epithelial-mesenchymal transition in human breast cancer cells. *Carcinogenesis* 2013; **34**: 864-873 [PMID: 23276794 DOI: 10.1093/carcin/bgs397]
29 **Scanlon CS**, Van Tubergen EA, Inglehart RC, D'Silva NJ. Biomarkers of epithelial-mesenchymal transition in squamous cell carcinoma. *J Dent Res* 2013; **92**: 114-121 [PMID: 23128109 DOI: 10.1177/0022034512467352]
30 **Vincent T**, Neve EP, Johnson JR, Kukalev A, Rojo F, Albanell J, Pietras K, Virtanen I, Philipson L, Leopold PL, Crystal RG, de Herreros AG, Moustakas A, Pettersson RF, Fuxe J. A SNAIL1-SMAD3/4 transcriptional repressor complex promotes TGF-beta mediated epithelial-mesenchymal transition. *Nat Cell Biol* 2009; **11**: 943-950 [PMID: 19597490 DOI: 10.1038/ncb1905]
31 **Achyut BR**, Yang L. Transforming growth factor-β in the gastrointestinal and hepatic tumor microenvironment. *Gastroenterology* 2011; **141**: 1167-1178 [PMID: 21839702 DOI: 10.1053/j.gastro.2011.07.048]

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BRIEF ARTICLE

Sustained virological response: A milestone in the treatment of chronic hepatitis C

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Abstract

AIM: To evaluate the long-term eradication of hepatitis C virus (HCV) infection and liver-related complications in chronically infected patients that have achieved sustained virological response.

METHODS: One hundred and fifty subjects with chronic hepatitis C (CHC) or cirrhosis and sustained virological response (SVR) between the years of 1989 and 2008 were enrolled in a long-term clinical follow-up study at the Gastrointestinal and Liver Unit of the University Hospital of Naples "Federico Ⅱ". At the beginning of the study, the diagnosis of HCV infection was made on the basis of serum positivity for antibodies to HCV and detection of HCV RNA transcripts, while a diagnosis of chronic hepatitis was formulated using imaging techniques and/or a liver biopsy. SVR was achieved by interferon-based therapy, both conventional and pegylated, with and without ribavirin treatment. The patients were evaluated for follow-up at a median length of 8.6 years, but ranged from 2-19.9 years. Among them, 137 patients had pre-treatment CHC and 13 had cirrhosis. The patients were followed with clinical, biochemical, virological, and ultrasound assessments on a given schedule. Finally, a group of 27 patients underwent a liver biopsy at the beginning of the study and transient elastography at their final visit to evaluate changes in liver fibrosis.

RESULTS: The median follow-up was 8.6 years (range 2-19.9 years). HCV RNA remained undetectable in all patients, even in patients who eventually developed liver-related complications, indicating no risk of HCV recurrence. Three liver-related complications were observed: two cases of hepatocellular carcinoma and one case of bleeding from esophageal varices resulting in an incidence rate of 0.23%/person per year. Further, all three complications took place in patients diagnosed with cirrhosis before treatment began. Only one death due to liver-related causes occurred, resulting in a mortality rate of 0.077% person per year. This amounts to a 99.33% survival rate in our cohort of patients after therapy for HCV infection. Finally, of the 27 patients who underwent a liver biopsy at the beginning of the study, a reduction in liver fibrosis was observed in 70.3% of the cases; only three cases registering values of liver stiffness indicative of significant fibrosis.

CONCLUSION: Patients with CHC and SVR show an excellent prognosis with no risk of recurrence and a very low rate of mortality. Our data indicate that virus-eradication following interferon treatment can last up to 20 years.

Key words: Antiviral therapy; Cirrhosis; Hepatitis C virus; Sustained virological response; Fibrosis

Core tip: This study represents one of the longest follow-up studies on the natural history of successfully treated chronically hepatitis C virus (HCV) infected individuals. The outcome of the study was very positive, as it revealed an extremely high survival rate, an extremely low rate of liver complications, and a significant reduction in liver fibrosis in patients who have achieved sustained virological response (SVR). All of the patients without cirrhosis before starting the treatment showed no signs of evolution or decompensation over the years of observation, proving that SVR positively changes the natural history in individuals with HCV-infection.

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INTRODUCTION

Hepatitis C virus (HCV) infection represents a serious challenge to global health, with an estimated 170 million carriers worldwide[1]. A significant complication in chronically infected individuals is the development of liver inflammation and fibrosis, which may progress to liver cirrhosis and hepatocellular carcinoma (HCC)[2-10].

For nearly 20 years, interferon-based therapy has been the standard therapy for chronic hepatitis C (CHC), with the optimal treatment of the combination of pegylated-interferon and ribavirin dating back ten years. The primary goal of HCV treatment is to achieve a sustained virological response (SVR). SVR is defined by undetectable HCV RNA levels 24 wk after completing treatment[11]. The achievement of the SVR in patients with CHC represents a milestone because it has been associated with the eradication of the infection, improvement in liver histology, improvement in quality of life, and reduced risk of cirrhosis and hepatocellular carcinoma[12-15]. Short term SVR in patients with chronic HCV-infection is well established, however, knowledge regarding long-term SVR remains to be elucidated. There are limited long-term studies observing chronically infected patients who have achieved SVR, all with a limited number of patients[12,16]. Further, studies that do have a large cohort of patients have a fairly short follow-up period[17,18]. Finally, the data gathered thus far lack real world examples as data gathered by subjects with SVR are usually limited to those participating in pivotal clinical trials.

The aim of this study was to evaluate the long-term outcomes of patients who obtain a SVR after antiviral therapy, regardless of the type treatment, in a monocentric cohort of patients representing a real world population with CHC during the last 20 years.

MATERIALS AND METHODS

Patients

From January 1989 to April 2008, 150 HCV-positive patients who achieved SVR after interferon-based therapy at the Gastroenterology Unit of the University of Naples "Federico Ⅱ" were enrolled in this cohort study. There were 100 men and 50 women (mean age, 56.37 ± 12.05 years; range 22-67 years). The main characteristics of our study population at baseline are reported in Table 1.

In this cohort, 137/150 (91.3%) patients had chronic hepatitis and 13 had liver cirrhosis. Before treatment, 21 patients had normal alanine transaminase (ALT) values and 129/150 (86%) had elevated ALT values ranging between 1.2 and 15.87 times the upper normal limit. A histological examination was performed in 103/150 (68.6%) of patients at baseline, while 47/150 (31.3%) patients refused the biopsy procedure and 27 patients showed comorbidities (Table 1). Nine patients had a diagnosis of arterial hypertension and two had an incomplete right bundle branch block. One patient had a medical history of previous angina pectoris and another suffered from a previous myocardial infarction, however, there was no cardiologic contraindication at the time the therapy started. Seven patients had thyroid disease; two patients had type Ⅱ diabetes; two had gastroesophageal reflux disease, and two had a co-infection of hepatitis B. One patient had a history of non-Hodgkin lymphoma that had been in remission for five years since the time the antiviral therapy started.

Modality of diagnosis

The diagnosis of HCV infection was made on the basis of serum positivity for antibodies to HCV and detection of HCV RNA transcripts. The diagnosis of chronic hepatitis was formulated by a histological assessment and scored according to the internationally accepted criteria[19]. The liver biopsy was not performed if the patients refused the procedure or had evidence of clinical, biochemical, ultrasound and endoscopic signs of liver cirrhosis. Transient elastography (TE), a non-invasive method of quantifying fibrosis developed as an alternative to liver biopsy, was carried out using FibroScan. Ultrasound elastography analyzes ultrasound frequency waves related to the elasticity (deforming capacity) of the liver. The methodology is simple, highly reproducible and can be completed in ten minutes. The diagnostic accuracy of TE is not as high as a liver biopsy, which remains the gold standard for evaluation of fibrosis. Nonetheless, in this context, TE is both highly sensitive and specific (79%-83% and 83%-89%, respectively)[20].

Therapy schedules

All patients were treated with interferon-based therapy,

Table 1 Baseline characteristics of 150 chronically infected hepatitis C virus patients with sustained virological response

Patients	150
Gender	
Male	100
Female	50
Patients with histological assessment[20]	103
Mild hepatitis (G0-G4)	52
Moderate hepatitis (G5-G9)	44
Severe hepatitis (G10-G18)	5
Staging S0-S1	44
Staging S2	25
Staging S3	10
Staging S4-S5	9
Cirrhosis S6	2
Genotype	
1a	14
1b	75
2	54
3	5
4	2
Comorbidity	
Cardiovascular diseases	13
Thyroid diseases	7
Crohn's disease	1
GERD	2
Diabetes mellitus type 2	2
HBV co-infection	2

GERD: Gastroesophageal reflux disease; HBV: Hepatitis B virus.

either in mono-therapy or in combination with ribavirin. Sixty-six subjects received conventional interferon mono-therapy. Twenty-five patients were treated with conventional interferon plus ribavirin and 59 patients were treated with pegylated interferon plus ribavirin. Conventional interferon was used at doses ranging between 3 and 6 MU every 2 d for a mean period of 49 ± 3.12 wk. The treatment regimen with pegylated interferon was carried out by international guideline recommendations and method indicated in the relevant product data sheets (Pegasys® 180 μg once weekly, subcutaneously; Pegintron® 1.5 μg/kg per week, subcutaneously; ribavirin 800, 1000 or 1200 mg/week orally, depending on body weight and virus genotype). The mean duration of the therapy was 47 ± 13 wk.

Follow-up

All patients were followed and analyzed for clinical, biochemical, virological and ultrasound parameters. All assessments were performed every 6 mo for the first two years of observation. In patients with cirrhosis at baseline, we continued to perform sequential clinical, biochemical and ultrasound follow-up every 6 mo, while the same panel of tests was conducted once a year in patients without cirrhosis. HCV RNA assessment was performed once per year during follow-up for all patients and whenever a decompensation or a progression of the liver disease occurred. At each medical visit, life-sign assessments and potential therapy-adverse effects were investigated. Biochemical evaluations measuring haemocrome (blood count), transaminases, bilirubin, alkaline phosphatase and renal function were performed. All patients underwent abdominal ultrasound every 6 mo. If a new space-occupying lesion was suspected, it was first analyzed by ultrasonography to determine if it was unquestionably benign (*e.g.*, cysts or hemangioma). If necessary, the lesion was further examined by computed tomography, magnetic resonance imaging, arteriography, or liver biopsy. In a group of 27 patients with liver biopsy at baseline, liver stiffness was assessed during the last visit. During this follow-up, cirrhotics were considered to have progression if they showed any of the following findings: ascites, bleeding varices, hepatic encephalopathy and HCC. The diagnosis of HCC was formulated using imaging techniques and/or biopsy, according to the Barcelona Clinical Liver Cancer standardized staging system for hepatocellular carcinoma[21].

Laboratory analysis

Serum was collected for detection of HCV RNA once per year after SVR was obtained. Qualitative detection of HCV-RNA was performed by a standardized qualitative assay, Cobas AmpliPrep/Cobas Taqman (CAP/CTM). This assay is based on the reverse transcription-polymerase chain reaction (RT-PCR) method, followed by HCV RNA real-time fluorescent detection from 850 μL of serum. HCV RNA concentration was read in IU/mL. The CAP/CTM assay has a sensitivity of 15 IU/mL, with a linear quantification range of 43-6.9 × 10^7 IU/mL.

RESULTS

Follow-up

The median duration of follow-up was 8.6 years (range 2-19.9 years). One hundred and fifteen/150 (76.6%) patients were followed for more than 4 years. Fifty-two/150 (34.6%) patients were followed for more than 10 years.

Virological outcomes

Zero of the 150 patients had a recurrence of HCV infection during the follow-up period. HCV RNA was evaluated in all patients with CAP/CTM assay, using at least four blood samples taken in different times (including the last sample available for each patient). No patient had detectable HCV-RNA in serum by RT-PCR in any sample, even in the patients who developed liver-related complications.

Survival and complication rates

The results from this study indicate the risk of liver-related death was only 0.67%, as only one patient died from liver-related causes. In addition, liver-related complications were observed in only three patients with a global complication incidence rate of 2%, while the complication incidence/person per year was 0.08%. These three complications included two patients with HCCs and one patient experiencing bleeding from esophageal varices. All three patients who developed complications had pre-treatment cirrhosis. Both patients who developed HCC were males, aged 61 developing the complication

Table 2 Liver fibrosis with paired assessment at baseline *vs* the end of follow-up

Baseline			End of follow-up		
Patients	METAVIR score	Corresponding value of LS (21) (kPa)	Mean value (range) of LS (kPa)	Mean follow-up (yr)	Patients with fibrosis regression
1	F0	< 6	5.9	10.6	0
18	F1	6.5 ± 1.1	5.4 (2.8-6.3)	9.48	13
6	F2	7.3 ± 1.4	6.2 (4.0-8.8)	6.02	4
0	F3	10.2 ± 1.9	-	-	-
1	F4	15 ± 4.1	6.8	8.3	1
1	Clinical cirrhosis	10.3	19.9	1	0

LS: Liver stiffness.

five years after follow-up, and aged 65 developing the complication nine years after follow-up, respectively. The 65-year-old male eventually died from liver-related complications. A 66-year-old female patient developed esophageal 17 bleeding years after the follow-up.

Regression of liver fibrosis

During the final visit, we performed TE on 27 patients who underwent a liver biopsy at baseline. The histological staging at baseline and the mean values of liver stiffness at the end of follow-up are reported in Table 2. Only in three cases were the values of liver stiffness higher than 7.3 kPa, the threshold indicative of significant fibrosis (F2 according to Metavir score). None of the patients had a liver stiffness score higher than 14.5 kPa, suggestive of cirrhosis. We observed regression of the fibrosis in 19/27 patients (70% of the cases).

DISCUSSION

The aim of this study was to assess the long-term effect of antiviral treatment in a large cohort of patients chronically infected with hepatitis C who had achieved SVR. This is one of the largest and longest studies on the natural history of successfully treated patients with chronic HCV infection in a real world setting. In the majority of the cases reported in the literature, the median follow-up duration is less than 5 years. In our study however, we observed 50 patients for up to 10 years and 21 patients for up to 15 years, with the median duration of the follow-up being 8.6 years. Overall, the clinical outcome of this study was very positive, indicating that prognosis in patients who obtained SVR is extremely promising.

The overall hepatic complication rate in our population was only 2%; a figure that is in agreement with other studies. Veldt *et al*[22] reported 3 cases of HCC in a population of 142 patients (2%) with SVR and a baseline fibrosis score that ranged between 4 and 6 according to the Ishak index[19]. Turner *et al*[23] reported 2 cases of HCC in a population of 152 patients (1.3%) with SVR and no cirrhosis. Ikeda *et al*[24] reported 30 cases of HCC in a population of 1097 patients (2.7%), of which 97 had cirrhosis and obtained SVR; but it was a retrospective, multicentric study with a median follow-up of 4.6 years. The high survival rate observed in our study (99.3%) is very consistent with values observed by George *et al*[18] (99.4%) and Imazeki *et al*[25] (99.97%).

We could not detect HCV-specific RNA transcripts in any of the patients at each follow-up, confirming that a durable SVR can be achieved with both conventional and pegylated interferon treatment[7-9]. Although a low rate of HCV-recurrence detected through RT-PCR assays has been reported, these data are in agreement with more recent studies on the topic[26-28]. The sensitivity of laboratory assays for HCV RNA detection has significantly increased throughout the years, and we can hypothesize that low serum levels of HCV RNA may not have been detected by a low sensitivity assay in the past, leading to a misclassification of SVR subjects. In fact, 5%, 9.7%, and 8% rates of HCV recurrence have been observed in previous studies[26-28]. In our study, HCV RNA was assessed through the most sensitive assay available (RT-PCR with sensitivity < 50 IU/mL). This sensitivity adds validity to our hypothesis that the hepatic complications observed in patients in our study were not directly related to ongoing HCV replication, but rather other complicating factors.

Of the 27 patients where fibrosis was evaluated, no patient with pre-treatment chronic hepatitis showed progression to cirrhosis, including those with advanced fibrosis (Ishak score 3-4/6). Moreover, a regression of fibrosis was reported in 70% of the cases. These results correlate well with the work by Poynard *et al*[29] showing that SVR (regardless of the therapy with which it is obtained) not only stops liver damage progression, but can also induce its regression. In this cohort, 1904 patients with CHC and SVR and with paired pre-treatment and post-treatment biopsies were observed; 86% of patients showed an improvement in fibrosis stage while 12% showed no change, even when the mean time between biopsies was only 20 mo[29]. Our data also comply with two recent studies with mean follow-up times of 5 and 6.5 years reporting 83% and 61% rates of fibrosis regression, respectively[18,30]. In these studies, the regression of fibrosis was assessed through liver biopsy. Although a liver biopsy is considered the gold standard; it does have limitations including sampling errors and interpretation of results influenced by intra- and inter-observer variation, implying that distinguishing real changes in fibrosis, longitudinally, is a difficult task[31]. Ellis *et al*[32] suggested that the most convincing evidence for regression of liver fibrosis derives from large-scale studies on post-treatment natural history. In fact, long-term follow-up studies indicate that regression of liver fibrosis is

associated with improved clinical outcomes, strengthening the perception that histological regression is a real phenomenon. Even though there is no universal parameter to define fibrosis regression, it is suggested that a long term assessment of clear clinical outcomes combined with non-invasive testing for fibrosis could help with the interpretation of the results[32].

In conclusion, our study documents that progression of liver damage and HCV infection relapse are virtually non-existent in patients with chronic hepatitis C infection who have achieved sustained virological response. Further, patients with pre-treatment evidence of cirrhosis show a rate of complications that cannot be neglected, but are hypothesized to occur due to complicating factors separate from HCV infection.

COMMENTS

Background

Sustained virological response (SVR) is by defined by undetectable hepatitis C virus (HCV) RNA levels 24 wk after completing treatment. The achievement of the SVR in patients with chronic hepatitis C (CHC) represents a milestone because it has been associated with the eradication of the infection, improvement in liver histology, improvement in quality of life, and reduced risk of cirrhosis and hepatocellular carcinoma. Short term SVR in patients with chronic HCV-infection is well established, however, knowledge regarding long-term SVR remains to be elucidated. There are limited long-term studies observing chronically infected patients who have achieved SVR, all with a limited number of patients.

Innovations and breakthroughs

This study represents one of the longest follow-up studies on the natural history of successfully treated chronically HCV infected individuals. The outcome of the study was very positive, as it revealed an extremely high survival rate, an extremely low rate of liver complications, and a significant reduction in liver fibrosis in patients who have achieved SVR.

Applications

The aim of this study was to evaluate the long-term outcomes of patients who obtain a SVR after antiviral therapy, regardless of the type treatment, in a monocentric cohort of patients representing a real world population with CHC during the last 20 years.

Peer review

It is a well planned study with sound methodology.

REFERENCES

1 **World Health Organization**. Hepatitis C- global prevalence (update). Available from: URL: http: //www.who.int/docstore/wer/pdf/2000/wer7503.pdf

2 **Nishiguchi S**, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, Shiomi S, Seki S, Kobayashi K, Otani S. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet* 1995; **346**: 1051-1055 [PMID: 7564784 DOI: 10.1016/S0140-6736(95)91739-X]

3 **Yoshida H**, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, Inoue O, Yano M, Tanaka M, Fujiyama S, Nishiguchi S, Kuroki T, Imazeki F, Yokosuka O, Kinoyama S, Yamada G, Omata M. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. *Ann Intern Med* 1999; **131**: 174-181 [PMID: 10428733]

4 **Poynard T**, Yuen MF, Ratziu V, Lai CL. Viral hepatitis C. *Lancet* 2003; **362**: 2095-2100 [PMID: 14697814 DOI: 10.1016/S0140-6736(03)15109-4]

5 **Yoshida H**, Tateishi R, Arakawa Y, Sata M, Fujiyama S, Nishiguchi S, Ishibashi H, Yamada G, Yokosuka O, Shiratori Y, Omata M. Benefit of interferon therapy in hepatocellular carcinoma prevention for individual patients with chronic hepatitis C. *Gut* 2004; **53**: 425-430 [PMID: 14960528 DOI: 10.1136/gut.2003.030353]

6 **Lavanchy D**. The global burden of hepatitis C. *Liver Int* 2009; **29** Suppl 1: 74-81 [PMID: 19207969 DOI: 10.1111/j.1478-3231.2008.01934.x]

7 **Bruno S**, Facciotto C. The natural course of HCV infection and the need for treatment. *Ann Hepatol* 2008; **7**: 114-119 [PMID: 18626427]

8 **Alter HJ**, Seeff LB. Recovery, persistence, and sequelae in hepatitis C virus infection: a perspective on long-term outcome. *Semin Liver Dis* 2000; **20**: 17-35 [PMID: 10895429 DOI: 10.1055/s-2000-9505]

9 **Ascione A**, Tartaglione T, Di Costanzo GG. Natural history of chronic hepatitis C virus infection. *Dig Liver Dis* 2007; **39** Suppl 1: S4-S7 [PMID: 17936222 DOI: 10.1016/S1590-8658(07)80003-X]

10 **Poynard T**, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; **349**: 825-832 [PMID: 9121257]

11 **Formann E**, Steindl-Munda P, Hofer H, Jessner W, Bergholz U, Gurguta C, Ferenci P. Long-term follow-up of chronic hepatitis C patients with sustained virological response to various forms of interferon-based anti-viral therapy. *Aliment Pharmacol Ther* 2006; **23**: 507-511 [PMID: 16441471 DOI: 10.1111/j.1365-2036.2006.02785.x]

12 **Tsuda N**, Yuki N, Mochizuki K, Nagaoka T, Yamashiro M, Omura M, Hikiji K, Kato M. Long-term clinical and virological outcomes of chronic hepatitis C after successful interferon therapy. *J Med Virol* 2004; **74**: 406-413 [PMID: 15368524 DOI: 10.1002/jmv.20190]

13 **Hung CH**, Lee CM, Lu SN, Wang JH, Hu TH, Tung HD, Chen CH, Chen WJ, Changchien CS. Long-term effect of interferon alpha-2b plus ribavirin therapy on incidence of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis. *J Viral Hepat* 2006; **13**: 409-414 [PMID: 16842444 DOI: 10.1111/j.1365-2893.2005.00707.x]

14 **Arase Y**, Ikeda K, Suzuki F, Suzuki Y, Saitoh S, Kobayashi M, Akuta N, Someya T, Koyama R, Hosaka T, Sezaki H, Kobayashi M, Kumada H. Long-term outcome after interferon therapy in elderly patients with chronic hepatitis C. *Intervirology* 2007; **50**: 16-23 [PMID: 17164553 DOI: 10.1159/000098958]

15 **Annicchiarico BE**, Siciliano M, Avolio AW, Grillo RL, Bombardieri G. A 5-year prospective study of the late resolution of chronic hepatitis C after antiviral therapy. *Aliment Pharmacol Ther* 2007; **25**: 1039-1046 [PMID: 17439504 DOI: 10.1111/j.1365-2036.2007.03295.x]

16 **Koh C**, Heller T, Haynes-Williams V. Clinical, virological, biochemical outcomes after 20 years of sustained virological response (SVR) in chronic hepatitis C: The NIH experience. 61th Annual Meeting of the American Association for the Study of Liver Diseases. Available from: URL: http: //www.natap.org/2010/AASLD/AASLD_25.htm

17 **Swain MG**, Lai MY, Shiffman ML, Cooksley WG, Zeuzem S, Dieterich DT, Abergel A, Pessôa MG, Lin A, Tietz A, Connell EV, Diago M. A sustained virologic response is durable in patients with chronic hepatitis C treated with peginterferon alfa-2a and ribavirin. *Gastroenterology* 2010; **139**: 1593-1601 [PMID: 20637202 DOI: 10.1053/j.gastro.2010.07.009]

18 **George SL**, Bacon BR, Brunt EM, Mihindukulasuriya KL, Hoffmann J, Di Bisceglie AM. Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. *Hepatology* 2009; **49**: 729-738 [PMID: 19072828 DOI: 10.1002/hep.22694]

19 **Ishak K**, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, Desmet V, Korb G, MacSween RN. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**: 696-699 [PMID: 7560864 DOI: 10.1016/0168-8278(95)80226-6]

20 **Tsochatzis EA**, Gurusamy KS, Ntaoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. *J Hepatol* 2011; **54**: 650-659 [PMID: 21146892 DOI: 10.1016/j.jhep.2010.07.033]

21 **Llovet JM**, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; **19**: 329-338 [PMID: 10518312 DOI: 10.1055/s-2007-1007122]

22 **Veldt BJ**, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, Zeuzem S, Manns MP, Hansen BE, Schalm SW, Janssen HL. Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. *Ann Intern Med* 2007; **147**: 677-684 [PMID: 18025443]

23 **Turner AJ**, Hick PE. Inhibition of aldehyde reductase by acidic metabolites of the biogenic amines. *Biochem Pharmacol* 1975; **24**: 1731-1733 [PMID: 16 DOI: 10.1080/00365520510015467]

24 **Ikeda M**, Fujiyama S, Tanaka M, Sata M, Ide T, Yatsuhashi H, Watanabe H. Risk factors for development of hepatocellular carcinoma in patients with chronic hepatitis C after sustained response to interferon. *J Gastroenterol* 2005; **40**: 148-156 [PMID: 15770398 DOI: 10.1007/s00535-004-1519-2]

25 **Imazeki F**, Yokosuka O, Fukai K, Saisho H. Favorable prognosis of chronic hepatitis C after interferon therapy by long-term cohort study. *Hepatology* 2003; **38**: 493-502 [PMID: 12883494 DOI: 10.1053/jhep.2003.50329]

26 **Marcellin P**, Boyer N, Gervais A, Martinot M, Pouteau M, Castelnau C, Kilani A, Areias J, Auperin A, Benhamou JP, Degott C, Erlinger S. Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and sustained response to interferon-alpha therapy. *Ann Intern Med* 1997; **127**: 875-881 [PMID: 9382365]

27 **Cammà C**, Di Marco V, Lo Iacono O, Almasio P, Giunta M, Fuschi P, Vaccaro A, Fabiano C, Magrin S, Di Stefano R, Bonura C, Pagliaro L, Craxì A. Long-term course of interferon-treated chronic hepatitis C. *J Hepatol* 1998; **28**: 531-537 [PMID: 9566819 DOI: 10.1016/S0168-8278(98)80274-2]

28 **Reichard O**, Glaumann H, Frydén A, Norkrans G, Wejstål R, Weiland O. Long-term follow-up of chronic hepatitis C patients with sustained virological response to alpha-interferon. *J Hepatol* 1999; **30**: 783-787 [PMID: 10365802 DOI: 10.1016/S0168-8278(99)80129-9]

29 **Poynard T**, McHutchison J, Manns M, Trepo C, Lindsay K, Goodman Z, Ling MH, Albrecht J. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2002; **122**: 1303-1313 [PMID: 11984517 DOI: 10.1053/gast.2002.33023]

30 **D'Ambrosio R**, Aghemo A, Rumi MG, Ronchi G, Donato MF, Paradis V, Colombo M, Bedossa P. A morphometric and immunohistochemical study to assess the benefit of a sustained virological response in hepatitis C virus patients with cirrhosis. *Hepatology* 2012; **56**: 532-543 [PMID: 22271347 DOI: 10.1002/hep.25606]

31 **Bedossa P**, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; **38**: 1449-1457 [PMID: 14647056 DOI: 10.1016/S0270-9139(03)80411-1]

32 **Ellis EL**, Mann DA. Clinical evidence for the regression of liver fibrosis. *J Hepatol* 2012; **56**: 1171-1180 [PMID: 22245903 DOI: 10.1016/j.jhep.2011.09.024]

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BRIEF ARTICLE

Long-term efficacy of endoscopic coagulation for different types of gastric vascular ectasia

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Abstract

AIM: To examine the long-term therapeutic efficacies of endoscopic cauterization for gastric vascular ectasia, according to the type of lesion.

METHODS: Thirty-eight patients with hemorrhagic gastric vascular ectasia (VE) were treated by endoscopic cauterization: 13 by heater probe coagulation- and 25 by argon plasma coagulation. Depending on the number of lesions, 14 and 24 patients were classified into localized VE (≤ 10; LVE) and extensive VE (> 10; EVE), respectively. The patients were followed-up by repeated endoscopic examinations after the therapy, and the incidences of VE recurrence and re-bleeding from the lesions were evaluated.

RESULTS: Although the VE lesions disappeared initially in all the patients after the therapy, the recurrence of VE developed in 25 patients (66%) over a mid-term observation period of 32 mo, and re-bleeding occurred in 15 patients (39%). The recurrence of VE was found in all patients with EVE, with re-bleeding occurring in 14 patients (58%). In contrast, only 1 patient (7%) with LVE showed recurrence of the lesions and complicating hemorrhage. Both the cumulative recurrence-free rates and cumulative re-bleeding-free rates were significantly lower in the EVE group than in the LVE group ($P < 0.001$ and $P < 0.001$, respectively). Moreover, the cumulative re-bleeding-free rate in the EVE group was 47.6% at 1 year and 25.4% at 2 years in patients with chronic renal failure, which were significantly lower than the rates in the patients without chronic renal failure (83.3% and 74.1%, respectively) ($P < 0.05$).

CONCLUSION: The recurrence of VE and re-bleeding from the lesions was more frequent in the patients with EVE, especially in those with complicating renal failure.

Key words: Gastric vascular ectasia; Heater probe coagulation; Argon plasma coagulation; Renal failure; Recurrence

Core tip: The aim of this study was to examine the long-term therapeutic efficacies of endoscopic cauterization for gastric vascular ectasia (VE), according to the type of lesion. Depending on the number of lesions, 14 and 24 patients were classified into localized vascular ectasia (≤ 10) and extensive VE (> 10; EVE), respectively. The incidences of VE recurrence and re-bleeding from the lesions were evaluated. The recurrence of VE and re-bleeding from the lesions was more frequent in the patients with EVE, especially in those with complicating renal failure, even after the initial successful arrest of bleeding and disappearance of the lesions by the endoscopic therapy.

Imai Y, Mizuno Y, Yoshino K, Watanabe K, Sugawara K, Motoya D, Oka M, Mochida S. Long-term efficacy of endoscopic

coagulation for different types of gastric vascular ectasia. *World J Gastroenterol* 2013; 19(18): 2799-2805 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i18/2799.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i18.2799

INTRODUCTION

Vascular ectasia (VE) of the stomach has been recognized as a rare cause of hemorrhage from the upper gastrointestinal tract[1-4]. Patients may present with either evidence of chronic occult bleeding, *i.e.*, iron deficiency anemia, or with acute hematemesis or melena. VE is a term that encompasses a broad spectrum of lesions visualized on endoscopic examination, including angiodysplasia, watermelon stomach, diffuse antral VE (DAVE) and telangiectasis associated with Osler-Weber-Rendu disease[5]. In general, angiodysplasia is defined as VE with a limited number of red flat spots or reticulated vascular areas in the gastric mucosa on endoscopic examination. In contrast, gastric antral VE (GAVE) is diagnosed as watermelon stomach when the lesions are visualized as stripes radiating outwards from the pylorus and as DAVE in cases with diffuse red spots in the gastric antrum.

Various therapeutic procedures have been employed for hemorrhagic gastric VE. Surgical resection of the stomach is the most curative therapeutic procedure, but it is a highly invasive procedure that maybe associated with high mortality, especially in elderly patients with underlying diseases such as metabolic syndrome. Thus, endoscopic cauterization has generally been performed for the treatment of gastric VE. Several types of coagulation procedures may be employed for endoscopic cauterization, including heater probe coagulation[6], laser coagulation[7-10] and argon plasma coagulation[10-16], and these devices have been reported to be useful for the temporary arrest of bleeding from any type of gastric VE. However, the long-term prognosis of patients undergoing endoscopic cauterization has to be elucidated. In the present paper, we examined the long-term therapeutic efficacies of endoscopic cauterization, including the recurrence rate of the lesions and the rates of re-bleeding, in patients with hemorrhaging from gastric VE, according to the type of lesion.

MATERIALS AND METHODS

Patients

The subjects included 42 patients with gastrointestinal bleeding from gastric VE lesions, which were diagnosed by gastrointestinal endoscopic examination between October 1996 and December 2007. Among them, 4 patients, who moved to a different institution for follow-up following discharge from our hospital after the successful arrest of bleeding from the VE, were excluded from the analysis. The remaining 38 patients consisted of 21 men and 17 women, with a median age of 69 years (range, 48-85 years). Twenty patients (53%) had underlying liver cirrhosis, and 15 patients (39%) were undergoing maintenance hemodialysis for chronic renal failure.

On endoscopic examination, bleeding from the gastric VE lesions was observed in 21 patients (55%). In the remaining 17 patients, other gastrointestinal lesions that could serve as the possible source of the bleeding were not detectable by the endoscopic examinations, including small intestinal endoscopy, despite the patient presenting with hematemesis or melena. The patients were classified into 2 groups: 14 patients with $\leqslant$ 10 VE lesions (localized VE, the LVE group) and 24 patients with > 10 VE lesions (extensive VE, the EVE group). The LVE group comprised 9 patients with a single lesion and 5 patients with multiple lesions (range, 3 to 10 lesions), including one patient with Osler-Weber-Rendu disease showing 3 lesions in the stomach. In contrast, the EVE group consisted of 5 patients with watermelon stomach and 19 patients with DAVE, including one patient with radiation-induced DAVE.

All patients were treated by endoscopic cauterization. Thereafter, follow-up endoscopic examinations were performed at intervals ranging from 1 to 6 mo, depending on the clinical features of each patient, and the rate of the recurrence of VE with or without re-bleeding from the lesions was evaluated. The recurrence of VE was defined as the presence of > 10 lesions in the EVE group, and of at least one lesion in the LVE group. The diagnosis of re-bleeding was conducted when the patients showed hematemesis or melena and/or hemorrhagic features of the gastric VE lesions on endoscopic examination irrespective of the number of lesions. Fifteen and twenty patients received treatment with a proton pump inhibitor and H_2-receptor antagonist, respectively, during the observation period. Written informed consent for the endoscopic procedures was obtained from all the patients. This study was retrospectively performed with the approval of the Institutional Review Board of the Hospital.

Endoscopic cauterization therapies

Thirteen patients seen between October 1996 and March 2000 were treated by heater probe coagulation, and twenty-five patients seen after April 2000 were treated by argon plasma coagulation. The therapies were repeated until the VE lesions completely disappeared from the stomach following the arrest of hemorrhaging. Heater probe coagulation was performed using a heater probe unit (Olympus Co., Tokyo, Japan) at 15 J for patients with bleeding VE and/or those with VE lesions with a diameter of > 3 mm and at 10 J for those with non-bleeding VE lesions with a diameter of $\leqslant$ 3 mm. Argon plasma coagulation was performed using a high-frequency generator (Erbotorm ICC200; ERBE, Tubingen, Germany) with an automatically regulated argon source (APC300; ERBE) and a flexible APC probe (ERBE) with a high-frequency output at 60 W. Argon gas was delivered at a flow rate of between 1.0 and 2.0 L/min.

Statistical analysis

The differences in the characteristics between the 2 groups

Table 1 Demographic and clinical features of the patients with gastrointestinal bleeding caused by gastric vascular ectasia

	Total	Groups[2]		*P* value
		LVE	EVE	
No. of patients	38	14	24	
Sex: male/female[1]	21 / 17	9/5	12/12	NS
Age, yr (medium)	69	67.5	72	NS
Bleeding from lesions at examination[1]	21	6	15	NS
Cauterization[3]				
Method : HPC/APC[1]	13 / 25	6/8	7/17	NS
No. of treatment sessions (mean ± SD)	2.3 ± 1.6	1.1 ± 0.3	3.0 ± 1.6	< 0.0001
Observation period, mo (medium)	32	68.5	29.5	< 0.01
Medication during observation[1]				
Proton pump inhibitors	15	4	11	NS
H_2-receptor antagonists	20	9	11	
None	3	1	2	
Underlying diseases[1]				
Chronic renal failure	15	3	12	NS
Liver cirrhosis	20	5	15	NS

[1]Number of the patients; [2]Localized vascular ectasia (LVE) denotes localized vascular ectasia and extensive vascular ectasia (EVE) denotes extensive vascular ectasia; [3]Heater probe coagulation (HPC) denotes heater probe coagulation and argon plasma coagulation (APC) denotes argon plasma coagulation. NS: Not significant.

were analyzed by Mann-Whitney's *U* test, Fisher's exact test and the χ^2 test. The cumulative recurrence-free and rebleeding-free rates were analyzed by the Kaplan-Meier method. Factors associated with the type of VE and the treatment methods were compared by the log-rank test. *P* values of less than 0.05 were considered to be statistically significant.

RESULTS

Demographic and clinical features of the patients in the LVE and EVE groups

As shown in Table 1, there were no significant differences in the sex distribution or age of the patients between the LVE and EVE groups. Hemorrhaging from the VE lesions was observed in 43% (6/14) and 63% (15/24) of the patients in the LVE and EVE groups, respectively. Additionally, 21% (3/14) and 36% (5/14) of the patients in the LVE group and 50% (12/24) and 63% (15/24) of the patients in the EVE group had underlying liver cirrhosis and chronic renal failure, respectively, but the prevalences of the 2 underlying diseases were not significantly different between the two groups.

Therapeutic efficacies of the endoscopic coagulation procedures

Among the 21 patients in whom active bleeding from the VE lesions was observed, heater probe coagulation was performed in 3 LVE and 4 EVE patients, and argon plasma coagulation in 3 LVE and 11 EVE patients. Hemostasis was obtained in all patients after either endoscopic coagulation procedure. Although the VE lesions diminished after the procedures in all patients, the number of treatment sessions required for the complete disappearance of the lesions in the stomach differed between the LVE and EVE groups (Table 1). A single treatment session was sufficient to achieve the complete disappearance of the VE lesions in all the patients of the LVE group, except one, who required 2 sessions of heater probe coagulation. In contrast, the patients in the EVE group needed a mean of 3.0 treatment sessions (range, 2-7 treatment sessions) for the complete disappearance of the lesions. No complications of the endoscopic coagulation treatments were encountered in either treatment group.

Table 2 Long-term efficacies of endoscopic cauterization therapies in the patients with gastrointestinal bleeding caused by gastric vascular ectasia *n* (%)

Groups	Recurrence rate	Re-bleeding rate
LVE	1 (7.1)	1 (7.1)
Without CRF	0 (0.0)	0 (0.0)
With CRF	1 (33.3)	1 (33.3)
EVE	24 (100.0)	14 (58.3)
Without CRF	12 (100.0)	6 (50.0)
With CRF	12 (100.0)	8 (66.7)

LVE: Localized vascular ectasia, EVE: Extensive vascular ectasia; CRF: Chronic renal failure.

In all patients, the recurrence of VE and of re-bleeding was examined by repeated follow-up endoscopic examinations for more than 6 mo after the final session of endoscopic cauterization therapy; the median observation period was 32 mo (range, 6-139 mo). As shown in Table 1, however, the observation periods were significantly longer in the LVE group than in the EVE group ($P < 0.01$). The medications prescribed for the patients during the observation period were similar between the two groups. The endoscopic examination revealed recurrence of the VE in 25 of 38 patients (66%), and re-bleeding from the recurrent gastric VE lesions in 15 of these patients (39%) over a median observation period of 5 mo (range, 2-70 mo) after the final session of the coagulation therapy. Particularly in the EVE group, the VE recurred in all the patients, and re-bleeding from the recurrent lesions developed in 14 of these patients (58%) (Table 2). No patients in the EVE group developed re-bleeding with the re-appearance of ≤ 10 VE lesions. All of the patients showing re-bleeding in the EVE group had underlying diseases: 8 with chronic renal failure, 5 with liver cirrhosis and 1 with radiation-induced mucosal damage of the gastrointestinal tract. In contrast, in the LVE group, the recurrence of the gastric VE was found in only 1 patient (7%), who had a single lesion that was found before the first session of argon plasma coagulation therapy. This patient also had underlying renal failure and was under long-term maintenance hemodialysis. Consequently, re-bleeding from the recurrent VE lesions developed in 60% (9/15) of the patients with chronic renal failure, which was significantly higher than the percentage in the patients without chronic renal failure (26%; 6/23) ($P = 0.036$).

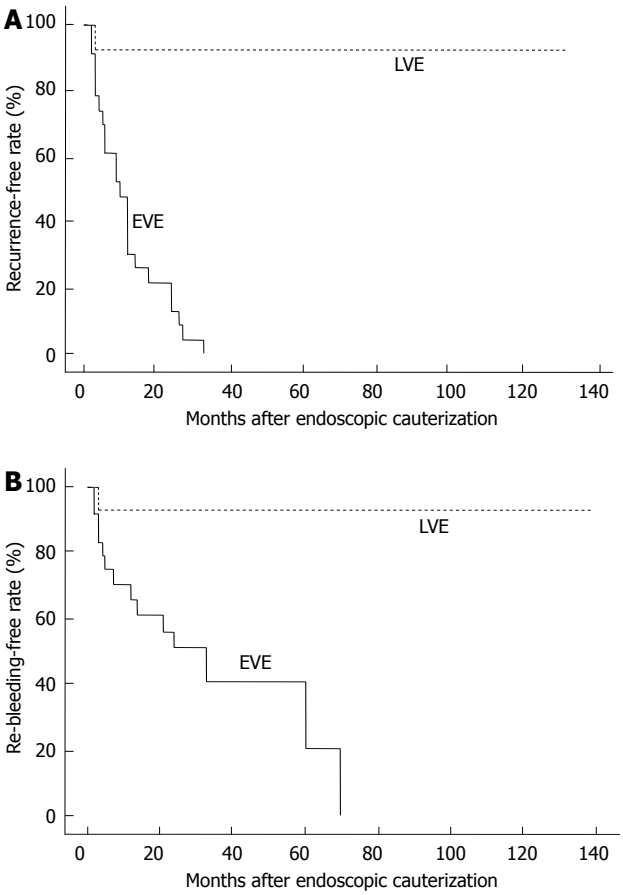

Figure 1 Long-term outcomes of the patients with hemorrhaging gastric vascular ectasia treated by endoscopic cauterization, depending on the number of lesions. A: Cumulative recurrence-free rates; B: Cumulative re-bleeding-free rates. Both rates were significantly higher in the localized vascular ectasia (LVE) group than in the extensive vascular ectasia (EVE) group ($P < 0.001$).

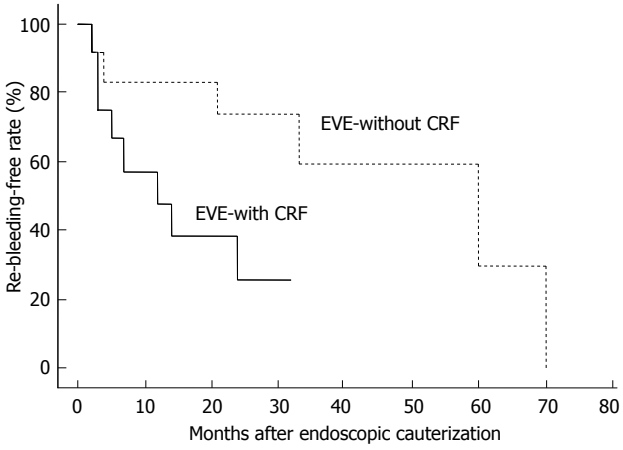

Figure 2 Cumulative re-bleeding-free rates in the patients with extensive vascular ectasia treated by endoscopic cauterization. The rates were significantly higher in the patients without chronic renal failure (CRF) than in those with CRF ($P < 0.05$). EVE: Extensive vascular ectasia.

Kaplan-Meier analysis revealed that the cumulative recurrence-free rates of the patients with VE were 29.2% and 0% at 1 and 3 years, respectively, after the endoscopic coagulation therapy in the EVE group (Figure 1A), and

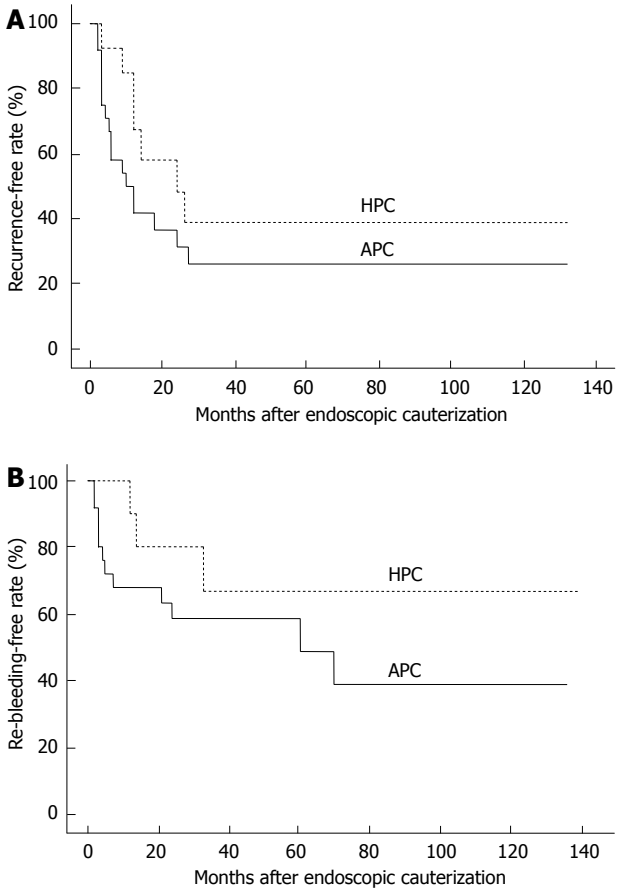

Figure 3 Long-term outcomes of the patients with hemorrhaging gastric vascular ectasia treated by endoscopic cauterization, depending on the type of procedure. A: Cumulative recurrence-free rates; B: Cumulative re-bleeding-free rates. Both the rates were comparable between the patients treated by heater probe coagulation (HPC) and those treated by argon plasma coagulation (APC).

the cumulative re-bleeding-free rates in these patients were 65.9%, 40.8% and 20.4% at 1, 3 and 5 years, respectively, after the therapy (Figure 1B). Both the recurrence-free rates and re-bleeding-free rates were significantly lower in the EVE group than in the LVE group. Moreover, the cumulative re-bleeding-free rate in the EVE group was 47.6% at 1 year and 25.4% at 2 years after the therapy in patients with chronic renal failure, which were significantly lower than the rates in the patients without chronic renal failure (83.3% and 74.1%, respectively) (Figure 2). There were no significant differences in either the recurrence-free rates or re-bleeding-free rates between the patients treated by heater probe coagulation and those treated by argon plasma coagulation, as shown in Figure 3.

DISCUSSION

In the present study, we examined the long-term prognosis of 38 patients with hemorrhagic gastric VE treated by endoscopic coagulation procedures, with a medium observation period of 32 mo after the therapies. Although both heater probe coagulation and argon plasma coagulation were useful to achieve the arrest of bleeding and

disappearance of the VE lesions, the recurrence of VE and re-bleeding from the recurrent lesions were noted in 66% and 39% of the patients, respectively. Additionally, we found that the recurrence of VE and/or re-bleeding from the recurrent lesions were more frequent in patients with EVE, especially those with underlying chronic renal failure undergoing maintenance hemodialysis, than in those with LVE. We also found that neither the lesion recurrence rate nor the re-bleeding rate differed significantly between the patients treated by heater probe coagulation and those treated by argon plasma coagulation, although the number of patients evaluated in the study was relatively small.

In Japan, endoscopic laser therapy has not been widely employed for the treatment of VE in the gastrointestinal tract because of the high risk of severe complications, such as perforation[8]. Thus, endoscopic cauterization either with heater probe coagulation or argon plasma coagulation is the standard therapeutic strategy used for gastric VE with or without bleeding. However, few studies have been performed to determine the long-term outcomes of such therapies. Olmos *et al*[13] reported that argon plasma coagulation was effective for the prevention of recurrent bleeding from VE in the gastrointestinal tract. Although they showed that re-bleeding did not occur from recurrent lesions in 83% of the patients over a median observation periods of 18 mo, only 10 patients with hemorrhaging gastric VE were included in their study after the exclusion of patients with chronic renal failure, liver cirrhosis and GAVE, including patients with the lesions caused by radiation. Moreover, Zushi *et al*[14] examined the outcomes in 16 patients with liver cirrhosis with hemorrhagic GAVE treated by endoscopic cauterization and reported that 25% of the patients showed re-bleeding from recurrent lesions. In their study, however, the mean observation period was only 10 mo, which is too short to evaluate the long-term outcomes of therapy in these patients. In contrast, Nakamura *et al*[15] evaluated the long-term efficacy of argon plasma coagulation in 22 patients with GAVE and reported that the cumulative re-bleeding rates at 1, 2 and 3 years after the therapy were 50.3%, 64.5% and 64.5%, respectively, over a mean observation period of 23.5 mo. Herrera *et al*[17] examined the therapeutic efficacy of argon plasma coagulation in patients with VE depending on the type of lesion, and reported that there were no cases of re-bleeding from recurrent lesions among the patients with LVE. Our results regarding the EVE group were in line with those reported by Nakamura *et al*[15] but not with those by Herrera *et al*[17], in which re-bleeding after the therapies developed only in 1 of 8 patients with GAVE. In our study, re-bleeding from recurrent VE was especially frequent in patients with EVE and in those with chronic renal failure undergoing maintenance hemodialysis, whereas underlying diseases such as chronic renal failure were found only in 3 patients with GAVE in the study by Herrera *et al*[17]. These differences in the clinical features of the patients may have produced the discrepancies in the results by Herrera *et al*[17], those by Nakamura *et al*[15] and those in the present study.

Both LVE and EVE have been reported to develop at a high frequency in association with chronic renal failure[18]. Clouse *et al*[3] reported that 18 of 30 patients (60%) with hemorrhagic angiodysplasia had underlying renal failure, with 10 of these patients (33%) under long-term maintenance hemodialysis and/or who underwent renal transplantation. The present study demonstrated that endoscopic cauterization did not provide satisfactory long-term outcomes in patients with EVE complicated with chronic renal failure, even after the successful initial arrest of bleeding had been achieved by the endoscopic cauterization therapy. Notably, the VE developed again after the therapies in all of the patients with EVE, even in the absence of underlying chronic renal failure, followed by re-bleeding from the recurrent lesions in half of these patients, with a cumulative re-bleeding rate of approximately 40% at 3 years. These observations prompted us to postulate that the etiology and the clinical characteristics may be different between the LVE and EVE groups, and also between VE patients with and without chronic renal failure. Based on the findings, careful endoscopic follow-up after the initial successful cauterization therapy is required for patients with EVE, regardless of the presence/absence of underlying diseases, including chronic renal failure.

With respect to the endoscopic cauterization procedures available, argon plasma coagulation has been employed more frequently compared with heater probe coagulation for the treatment of gastric VE in Japan. Similarly, at our institution, almost all patients seen after the year 2000 have been treated by argon plasma coagulation. As shown in Figure 3, both the cumulative recurrence and re-bleeding rates were equivalent between patients with gastric VE treated by argon plasma coagulation and those treated by heater probe coagulation. The limitation of our evaluation is that it is a retrospective and single cohort study. A randomized controlled study would be required to confirm our results. Additionally, the safety, including the frequency of complications, convenience of instrument handling, and number of sessions required for the treatment, has been reported to not be significantly different between the patients treated by the two procedures[19-22]. Thus, the criteria for the selection of either procedure for patients with gastric ectasia need to be established in the future. Recently, endoscopic band ligation was shown to be useful for the treatment of GAVE[23-27]. Wells *et al*[25] reported that endoscopic band ligation was superior to thermal therapies, including argon plasma coagulation, in terms of the therapeutic efficacy to arrest bleeding, the volume of blood transfusion needed after the procedure and the duration of hospitalization in 22 patients with GAVE. Sato *et al*[26] also reported the superiority of endoscopic band ligation-compared with argon plasma coagulation for GAVE as-

sociated with liver diseases. Moreover, a novel endoscopic ablation method, the HALO[90] system, has been reported to be useful for the treatment of GAVE in a few patients. A large-scale study would be required to compare the efficacy and safety of these novel procedures with those of argon plasma coagulation and heater probe coagulation.

In conclusion, the recurrence of VE and re-bleeding from the lesions was frequent in patients with EVE, especially in those with underlying chronic renal failure after the initial successful control of the bleeding and disappearance of the lesions by endoscopic cauterization. Therefore, careful observation by endoscopy is important for these patients even after initial successful therapy.

COMMENTS

Background

Vascular ectasia (VE) of the stomach has been recognized as a rare cause of hemorrhage from the upper gastrointestinal tract. Endoscopic coagulation has been reported to be useful for the temporary arrest of bleeding from any type of gastric VE. However, the long-term prognosis of patients undergoing endoscopic coagulation has not yet been elucidated.

Innovations and breakthroughs

The recurrence of VE and re-bleeding from the lesions was frequent in patients with extensive VE, especially in those with underlying chronic renal failure, after the initial successful control of the bleeding and disappearance of the lesions by endoscopic cauterization.

Applications

The findings of this study may help establish the treatment and follow-up strategy for the patients with bleeding gastric VE.

Terminology

VE is a term encompassing a broad spectrum of lesions visualized on endoscopic examination, including angiodysplasia, watermelon stomach and diffuse antral VE. In this study, VE lesions were classified into 2 subtypes: localized VE with $\leqslant$ 10 VE lesions and extensive VE with > 10 VE lesions.

Peer review

This is a paper on the treatment of VE of the stomach with some novel findings. The cohort is large and well described. It is worth publishing to demonstrate the novel finding of worse outcomes in those patients with chronic renal failure.

REFERENCES

1 **Wheeler MH**, Smith PM, Cotton PB, Evans DM, Lawrie BW. Abnormal blood vessels in the gastric antrum: a cause of upper-gastrointestinal bleeding. *Dig Dis Sci* 1979; **24**: 155-158 [PMID: 311727]

2 **Jabbari M**, Cherry R, Lough JO, Daly DS, Kinnear DG, Goresky CA. Gastric antral vascular ectasia: the watermelon stomach. *Gastroenterology* 1984; **87**: 1165-1170 [PMID: 6332757]

3 **Clouse RE**, Costigan DJ, Mills BA, Zuckerman GR. Angiodysplasia as a cause of upper gastrointestinal bleeding. *Arch Intern Med* 1985; **145**: 458-461 [PMID: 3872107 DOI: 10.1001/archinte.1985.00360030098019]

4 **Jensen DM**. Current diagnosis and treatment of severe obscure GI hemorrhage. *Gastrointest Endosc* 2003; **58**: 256-266 [PMID: 12872101 DOI: 10.1067/mge.2003.332]

5 **Clouse RE**. Vascular lesions: ectasias, tumours and malformations. In: Yamada T, editor. Textbook of Gastroenterology. 3rd ed. Philadelphia: Lippincott Williams and Wilkins, 1999: 2564-2582

6 **Petrini JL**, Johnston JH. Heat probe treatment for antral vascular ectasia. *Gastrointest Endosc* 1989; **35**: 324-328 [PMID: 2788591]

7 **Gostout CJ**, Ahlquist DA, Radford CM, Viggiano TR, Bowyer BA, Balm RK. Endoscopic laser therapy for watermelon stomach. *Gastroenterology* 1989; **96**: 1462-1465 [PMID: 2785467]

8 **Naveau S**, Aubert A, Poynard T, Chaput JC. Long-term results of treatment of vascular malformations of the gastrointestinal tract by neodymium YAG laser photocoagulation. *Dig Dis Sci* 1990; **35**: 821-826 [PMID: 2364836 DOI: 10.1007/BF01536794]

9 **Sargeant IR**, Loizou LA, Rampton D, Tulloch M, Bown SG. Laser ablation of upper gastrointestinal vascular ectasias: long term results. *Gut* 1993; **34**: 470-475 [PMID: 8491392 DOI: 10.1136/gut.34.4.470]

10 **Pavey DA**, Craig PI. Endoscopic therapy for upper-GI vascular ectasias. *Gastrointest Endosc* 2004; **59**: 233-238 [PMID: 14745397]

11 **Wahab PJ**, Mulder CJ, den Hartog G, Thies JE. Argon plasma coagulation in flexible gastrointestinal endoscopy: pilot experiences. *Endoscopy* 1997; **29**: 176-181 [PMID: 9201466]

12 **Yusoff I**, Brennan F, Ormonde D, Laurence B. Argon plasma coagulation for treatment of watermelon stomach. *Endoscopy* 2002; **34**: 407-410 [PMID: 11972274]

13 **Olmos JA**, Marcolongo M, Pogorelsky V, Varela E, Dávolos JR. Argon plasma coagulation for prevention of recurrent bleeding from GI angiodysplasias. *Gastrointest Endosc* 2004; **60**: 881-886 [PMID: 15605001]

14 **Zushi S**, Imai Y, Fukuda K, Yabuta T, Tsujino S, Yamada T, Kurokawa M. Endoscopic coagulation therapy is useful for improving encephalopathy in cirrhotic patients with gastric antral vascular ectasia. *Digest Endosc* 2005; **17**: 32-35 [DOI: 10.1111/j.1443-1661.2005.00426.x]

15 **Nakamura S**, Mitsunaga A, Konishi H, Oi I, Shiratori K, Suzuki S. Long-term follow up of gastric antral vascular ectasia treated by argon plasma coagulation. *Digest Endosc* 2006; **18**: 128-133 [DOI: 10.1111/j.0915-5635.2006.00592.x]

16 **Lecleire S**, Ben-Soussan E, Antonietti M, Goria O, Riachi G, Lerebours E, Ducrotté P. Bleeding gastric vascular ectasia treated by argon plasma coagulation: a comparison between patients with and without cirrhosis. *Gastrointest Endosc* 2008; **67**: 219-225 [PMID: 18226684 DOI: 10.1016/j.gie.2007.10.016]

17 **Herrera S**, Bordas JM, Llach J, Ginès A, Pellisé M, Fernández-Esparrach G, Mondelo F, Mata A, Cárdenas A, Castells A. The beneficial effects of argon plasma coagulation in the management of different types of gastric vascular ectasia lesions in patients admitted for GI hemorrhage. *Gastrointest Endosc* 2008; **68**: 440-446 [PMID: 18423466]

18 **Cunningham JT**. Gastric telangiectasias in chronic hemodialysis patients: a report of six cases. *Gastroenterology* 1981; **81**: 1131-1133 [PMID: 6974673]

19 **Protell RL**, Rubin CE, Auth DC, Silverstein FE, Terou F, Dennis M, Piercey JR. The heater probe: a new endoscopic method for stopping massive gastrointestinal bleeding. *Gastroenterology* 1978; **74**: 257-262 [PMID: 620899]

20 **Imai Y**, Kinoshita M, Asakura Y, Kakinuma T, Arai S, Shimoji K, Sasaki K, Yabe S, Ota S, Fujiwara K. Usefulness of heater probe therapy for non-ulcer lesion. *Prog Dig Endosc* 1999; **54**: 40-42

21 **Grund KE**, Storek D, Farin G. Endoscopic argon plasma coagulation (APC) first clinical experiences in flexible endoscopy. *Endosc Surg Allied Technol* 1994; **2**: 42-46 [PMID: 8081915]

22 **Asakura Y**, Imai Y, Arai S, Kinoshita M, Kakinuma T, Kakoi K, Rai F, Eguchi Y, Fujiwara K, Ota S. Efficacy of argon plasma coagulation for bleeding gastroduodenal ulcers. *Digest Endosc* 2002; **14**: 99-102 [DOI: 10.1046/j.1443-1661.2002.00186.x]

23 **Sinha SK**, Udawat HP, Varma S, Lal A, Rana SS, Bhasin DK. Watermelon stomach treated with endoscopic band ligation. *Gastrointest Endosc* 2006; **64**: 1028-1031 [PMID: 17140926]

24 **Kumar R**, Mohindra S, Pruthi HS. Endoscopic band ligation: a novel therapy for bleeding gastric antral vascular ectasia. *Endoscopy* 2007; **39** Suppl 1: E56-E57 [PMID: 17323279]

25 **Wells CD**, Harrison ME, Gurudu SR, Crowell MD, Byrne TJ, Depetris G, Sharma VK. Treatment of gastric antral

vascular ectasia (watermelon stomach) with endoscopic band ligation. *Gastrointest Endosc* 2008; **68**: 231-236 [PMID: 18533150 DOI: 10.1016/j.gie.2008.02.021]

26 **Sato T**, Yamazaki K, Akaike J. Endoscopic band ligation versus argon plasma coagulation for gastric antral vascular ectasia associated with liver diseases. *Dig Endosc* 2012; **24**: 237-242 [PMID: 22725108 DOI: 10.1111/j.1443-1661.2011.01221.x]

27 **Gross SA**, Al-Haddad M, Gill KR, Schore AN, Wallace MB. Endoscopic mucosal ablation for the treatment of gastric antral vascular ectasia with the HALO90 system: a pilot study. *Gastrointest Endosc* 2008; **67**: 324-327 [PMID: 18226696 DOI: 10.1016/j.gie.2007.09.020]

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BRIEF ARTICLE

Extremely high prevalence of *Helicobacter pylori* infection in Bhutan

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Abstract

AIM: To revealed the prevalence of *Helicobacter pylori* (*H. pylori*) infection in the Bhutanese population.

METHODS: We recruited a total of 372 volunteers (214 females and 158 males; mean age of 39.6 ± 14.9 years) from three Bhutanese cities (Thimphu, Punaka, and Wangdue). The status of *H. pylori* infection was determined based on five different tests: the rapid urease test (CLO test), culture, histology, immunohistochemistry (IHC), and serum anti *H. pylori*-antibody.

RESULTS: The serological test showed a significantly higher positive rate compared with the CLO test, culture, histology and IHC ($P < 0.001$, $P < 0.001$, $P = 0.01$, and $P = 0.01$, respectively). When the subjects were considered to be *H. pylori* positive in the case of at least one test showing a positive result, the overall prevalence of *H. pylori* infection in Bhutan was 73.4%. The prevalence of *H. pylori* infection significantly decreased with age ($P < 0.01$). The prevalence of *H. pylori* infection was lower in Thimphu than in Punakha and Wangdue ($P = 0.001$ and 0.06, respectively). The prevalence of *H. pylori* infection was significantly higher in patients with peptic ulcers than in those with gastritis (91.4% *vs* 71.3%, $P = 0.003$).

CONCLUSION: The high incidence of gastric cancer in Bhutan may be attributed to the high prevalence of *H. pylori* infection.

Key words: *Helicobacter pylori*; Bhutan; Prevalence

Core tip: The high prevalence of *Helicobacter pylori* (*H. pylori*) infection in Bhutan may contribute to the high incidence of peptic ulcers and gastric cancer. The prevalence of *H. pylori* infection in the capital city, Thimphu, was significantly lower than that of other rural areas. Therefore, performing eradication therapy of *H. pylori* and improving the sanitary conditions to decrease the rate of *H. pylori* infection in Bhutan can contribute to decreasing *H. pylori*-related diseases such as peptic ulcers and gastric cancer.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a spiral, Gram-negative bacterium that chronically colonizes the human stomach and is currently recognized to play a causative role in the pathogenesis of various gastroduodenal diseases, including gastritis, peptic ulcers, gastric cancer, and mucosa-associated lymphoid tissue lymphoma[1,2]. Infection with *H. pylori* almost always results in chronic gastritis, but only a small population of infected patients develop more severe diseases, such as peptic ulcers and gastric cancer[2,3]. In Asia, gastric cancer is still a significant health problem, and the incidence of gastric cancer geographically varies greatly. Based on the age-standardized incidence rate (ASR) of gastric cancer, Asian countries can be categorized as high-risk (*e.g.*, Japan, South Korea and China), intermediate-risk (*e.g.*, Vietnam) or low-risk (*e.g.*, Thailand and Indonesia) countries for gastric cancer[4]. Although the association between *H. pylori* infection and gastric cancer has been well-established[5,6], a high prevalence of *H. pylori* infection is not always associated with a high incidence of gastric cancer. For example, despite the high infection rate in India, the incidence of gastric cancer there is low, which is known as an "Asian enigma"[7].

Bhutan is a small landlocked country in South Asia, located at the eastern end of the Himalayas and bordered on the South, East and West by India and on the North by China. The ASR of gastric cancer in Bhutan was reported to be 24.2/100000, which is relatively high among Asian countries[4]. Although several studies focused on the prevalence of *H. pylori* infection have been conducted in many countries with different socioeconomic, cultural, and racial groups[8-10], the prevalence of *H. pylori* infection in Bhutan has not been investigated yet. In this study, we first disclosed the infection rate of *H. pylori* in Bhutan, and the findings from this study can be used as baseline epidemiological data for further research to understand the epidemiology of *H. pylori* infection in Bhutan and other Asian countries.

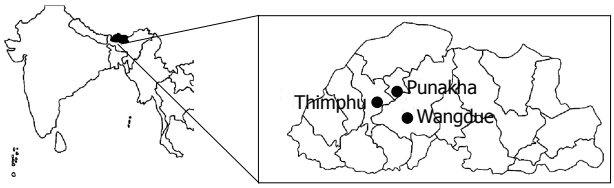

Figure 1 Geographic location in Bhutan.

MATERIALS AND METHODS

Study population

We recruited a total of 372 volunteers with dyspeptic symptoms (214 females and 158 males; mean age of 39.6 ± 14.9 years) during four days (December 6 to 9) in December 2010. The survey was conducted in Thimphu (n = 194), the capital city, and in two other cities within a 200 km radius of the capital, Punakha (n = 119) and Wangdue (n = 59) (Figure 1). Written informed consent was obtained from all participants, and the protocol was approved by the Ethics Committee of Jigme Dorji Wangchuk National Referral Hospital, Bhutan.

During each endoscopy session, four gastric biopsy specimens were obtained (three from the antrum and one from the corpus). The three specimens from the antrum were used for *H. pylori* culture, rapid urease test and histological examination. The specimen from the corpus was used for histological examination. Peptic ulcers and gastric cancer were identified by endoscopy, and gastric cancer was further confirmed by histopathology. Gastritis was defined as *H. pylori* gastritis in the absence of a peptic ulcer or gastric malignancy.

Status of H. pylori infection

To maximize the diagnostic accuracy, 5 different methods were combined for the diagnosis of *H. pylori* infection, including culture, histology, immunohistochemistry (IHC), the rapid urease test and serum *H. pylori* antibody. For the *H. pylori* culture, one biopsy specimen from the antrum was homogenized in saline and inoculated onto Mueller Hinton Ⅱ Agar medium (Becton Dickinson, NJ, United States) supplemented with 7% horse blood without antibiotics. The plates were incubated for up to 10 d at 37 ℃ under microaerophilic conditions (10% O_2, 5% CO_2 and 85% N_2). *H. pylori* was identified on the basis of colony morphology, Gram staining and positive reactions for oxidase, catalase, and urease. Isolated strains were stored at -80 ℃ in Brucella Broth (Difco, NJ, United States) containing 10% dimethylsulfoxide and 10% horse serum. For histology, all biopsy materials were fixed in 10% buffered formalin for 24 h and then embedded in paraffin. Serial sections were stained with hematoxylin and eosin and with May-Giemsa stain. The state of the gastric mucosa was evaluated according to the updated Sydney system[11]. The degree of the bacterial load was classified into four grades: 0, "normal"; 1, "mild"; 2, "moderate"; and 3, "marked". A bacterial load grade greater than or equal to 1 was defined as *H. pylori* positive. *H. pylori* seropositivity was evaluated with a commercially available ELISA kit (Eiken Co., Ltd.,

Table 1 Prevalence of *Helicobacter pylori* infection in each diagnostic test *n* (%)

Diagnostic test	Age (yr)					Total (*n* = 372)
	≤ 29 (*n* = 107)	30-39 (*n* = 96)	40-49 (*n* = 80)	50-59 (*n* = 45)	≥ 60 (*n* = 44)	
Serum	80 (74.8)	80 (83.3)	47 (58.8)	30 (66.7)	24 (54.5)	261 (70.2)
CLO	68 (63.6)	59 (61.5)	35 (43.8)	24 (53.3)	17 (38.6)	203 (54.6)
Culture	72 (67.3)	61 (63.5)	38 (47.5)	23 (51.1)	16 (36.4)	210 (56.5)
Histology	77 (72.0)	64 (66.7)	41 (51.3)	28 (62.2)	19 (43.2)	229 (61.6)
IHC	77 (72.0)	64 (66.7)	41 (51.3)	28 (62.2)	19 (43.2)	229 (61.6)
Final	86 (80.4)	80 (83.3)	49 (61.3)	32 (71.1)	26 (59.1)	273 (73.4)

CLO: The rapid urease test; IHC: Immunohistochemistry.

Tokyo, Japan) according to the manufacturer's instructions. Patients were considered to be *H. pylori*-negative when all five tests were negative, and a *H. pylori*-positive status required at least one positive test result.

Immunohistochemistry

IHC was performed as described previously[12]. Briefly, after antigen retrieval and inactivation of endogenous peroxidase activity, tissue sections were incubated with the α-*H. pylori* Ab (DAKO, Denmark) overnight at 4 ℃. After washing, the sections were incubated with biotinylated goat anti-rabbit IgG (Nichirei Co., Japan), followed by incubation with a solution of avidin-conjugated horseradish peroxidase (Vectastain Elite ABC kit; Vector Laboratories Inc., Burlingame, CA, United States). Peroxidase activity was detected using a H_2O_2/diaminobenzidine substrate solution. For all cases, we performed Giemsa staining using a serial section to identify the presence of *H. pylori*. If the *H. pylori* identified by Giemsa staining was found to be positively immunostained, we judged the case to be positive.

Statistical analysis

The statistical analysis were conducted using the chi-square test to compare discrete variables and Cochran-Armitage analysis to compare the prevalence of *H. pylori* infection. Differences in the prevalence in each group were analyzed using the Mantel-Haenszel method. To match age and sex, multiple backward stepwise logistic regression analyses were used to examine the associations of peptic ulcers with the main predictor variables. The predictor variables for peptic ulcers were age, sex and *H. pylori* status. For each variable, the OR and 95%CI were calculated. Differences at $P < 0.05$ were regarded as statistically significant. The data analysis was performed using JMP® 9 statistical software (SAS Institute Inc., Cary, NC, United States) and SPSS version 19 (SPSS Inc., Chicago, IL, United States).

RESULTS

A total of 372 subjects were recruited, comprising 107 who were ≤ 29 years old, 96 who were 30-39 years old, 80 who were 40-49 years old, 45 who were 50-59 years old, and 44 who were ≥ 60 years old. Table 1 shows the *H. pylori*-positive rate for each test. The serological test showed a significantly higher positive rate compared with the CLO test, culture, histology and IHC ($P < 0.001$, $P < 0.001$, $P = 0.01$, and $P = 0.01$, respectively). The prevalence of *H. pylori* infection by the serological test was as follows: 74.8% (80/107) for the ≤ 29 years old group, 83.3% (80/96) for the 30-39 years old group, 58.8% (47/80) for the 40-49 years old group, 66.7% (30/45) for the 50-59 years old group, and 54.5% (24/44) for the ≥ 60 years old group. When the subjects were considered to be *H. pylori* positive in the case of at least one positive test, the prevalence of *H. pylori* was 80.4% (86/107) for the ≤ 29 years old group, 83.3% (80/96) for the 30-39 years old group, 61.3% (49/80) for the 40-49 years old group, 71.1% (32/45) for the 50-59 years old group, and 59.1% (26/44) for the ≥ 60 years old group; thus, the prevalence was significantly decreased with age ($P < 0.01$). This phenomenon was significant in the Thimphu area. Because the number of subjects older than 50 years old in Punakha and Wangdue was too small for the statistical analysis, this trend was not observed in these two areas. Overall, the prevalence of *H. pylori* infection in Bhutan was 73.4% (273/372). Figure 2 shows the prevalence of *H. pylori* infection according to the various range age groups. There was no significant difference between men and women (data not shown).

The prevalence of *H. pylori* infection in the three cities was analyzed by the Mantel-Haenszel method to adjust for age. It differed among the three cities, with the highest in Punakha (102/119, 85.7%), followed by Wangdue (44/59, 74.5%) and Thimphu (127/194, 65.4%). The prevalence of *H. pylori* infection was significantly lower in Thimphu than in Punakha even after the adjustment for age ($P = 0.001$). Although there was no significant difference, the prevalence tended to be lower in Thimphu than in Wangdue even after the adjustment for age ($P = 0.06$).

In the endoscopic diagnosis, gastritis was the most common finding (307/372, 82.5%). Gastric and duodenal ulcers were found in 25 (6.7%) and 22 (5.9%) cases, respectively. Gastric cancer was found in 5 cases (1.3%). Duodenal erosion, duodenal tumor, and reflux esophagitis were found at 5, 1 and 7 cases, respectively. Table 2 shows the prevalence *H. pylori* infection in each diagnosis. A high infection rate was detected among patients with gastric ulcers (92.0%) and duodenal ulcers (90.9%). In addition, 71.3% of the subjects with gastritis were *H. pylori*-positive. When gastric and duodenal ulcers were defined as peptic ulcers, the prevalence of *H. pylori* infection in peptic ulcers was significantly higher than that in gastritis (91.4% *vs* 71.3%, $P = 0.003$). The percentage of

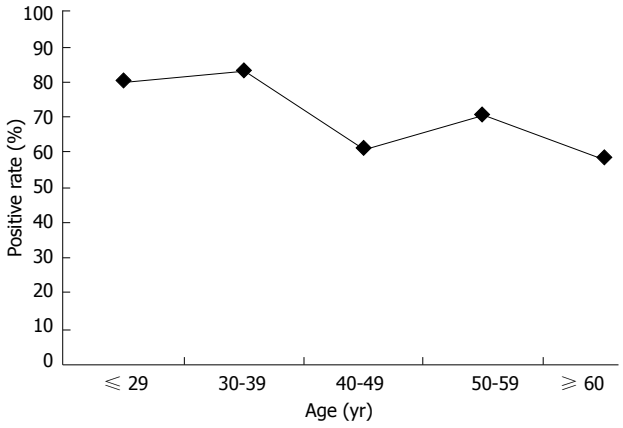

Figure 2 Prevalence of *Helicobacter pylori* infection by age group in Bhutan.

men was also significantly higher in patients with peptic ulcers than in those with gastritis (72.3% *vs* 37.1%, $P < 0.001$). Multiple logistic analysis after adjusting for age and gender showed that *H. pylori*-positivity in addition to male gender was significantly associated with peptic ulcers (OR = 3.89, 95%CI: 1.33-11.33).

DISCUSSION

We first revealed that the prevalence of *H. pylori* in Bhutan was 73.4%. In contrast with developed countries, *H. pylori* infections occur earlier in life and with a higher frequency in the developing world[8]. The prevalence of infection with *H. pylori* infection exceeds 50% by 5 years of age, and by adulthood, infection rates exceeding 90% are not unusual in developing countries[13]. Although the prevalence of infection has dropped significantly in many parts of North America, Western Europe and Asia, no such decline has been noted in the developing world[13]. The present study showed that a high prevalence was detected in younger age groups and that the prevalence significantly decreased with age in Bhutan. The decrease in the *H. pylori* infection rate with age might be due to the overuse of antibiotics in Bhutan. Antibiotics are frequently used in any infectious diseases in Bhutan (Dr. Lotay, personal communication). Even under these conditions, the prevalence of *H. pylori* infection was relatively high in all age groups.

Several clinical tests have been developed to diagnose *H. pylori* infection. However, there is still no established "gold standard" for the diagnosis of *H. pylori*, and thus the combination of two or more tests should be applied to determine the accurate prevalence of infection. In this study, we combined 5 different tests and considered *H. pylori*-positivity to be at least one positive test among the five tests. In this study, the serological test showed the highest positive rate compared with the other 4 tests. Although the serological test is widely used in epidemiological studies and not affected by local changes in the stomach that could lead to false-negatives, as in the other tests, this test cannot distinguish between current and past infections because *H. pylori* IgGs persist even after the disappearance of this bacterium[9,14]. Therefore,

Table 2 Prevalence of *Helicobacter pylori* infection in each diagnosis *n* (%)

Diseases	*n*	Positive
Gastritis	307	219 (71.3)
Gastric ulcer	25	23 (92.0)
Duodenal ulcer	22	20 (90.9)
Gastric cancer	5	3 (60.0)
Duodenal erosion	5	3 (60.0)
Duodenal tumor	1	1 (100.0)
Reflux esophagitis	7	4 (57.1)

results that are positive in the serological test and negative in the endoscopic tests may indicate a past infection. Culture from biopsy specimens has the potential of leading to a high sensitivity, given that only one bacterium can multiply and provide billions of bacteria. However, both strict transport conditions and careful handling in the laboratory are necessary[14]. Histopathological positivity depends on the density of *H. pylori* biopsy sites; thus, these tests can occasionally show false negative results[14]. In addition, the histological diagnosis of *H. pylori* infection is very much dependent on the expertise of the pathologists. The rapid urease test, such as the CLO test, can be useful as a rapid diagnostic method. However, these results can also be affected by the bacterial load[14]. A high proportion of the elderly population develops gastric atrophy and intestinal metaplasia, which can lead to a hostile environment for *H. pylori* and thus fewer bacteria and potentially a negative result. A detailed study in histological scoring is necessary for further study. Moreover, endoscopic tests, including the CLO test, culture and histological examination, can be affected in bleeding patients with peptic ulcers[10]. However, although the reason is not clear, all peptic ulcer cases in our study were not in the active bleeding phase, indicating that we do not need to consider any effects of bleeding.

The lowest infection rate was found in Thimphu, which is the capital city of Bhutan. The prevalence of *H. pylori* infection in Thimphu was significantly lower than that of the rural cities of Punakha and Wangdue. In Thimphu, the sanitary conditions are better than those of Punakha and Wangdue, which supports the possibility that sanitary conditions may be important factors for *H. pylori* infection. In fact, the prevalence of *H. pylori* infection was very low in individuals less than 10 years old (*i.e.*, approximately 5%) and increased with age in Japan[15]. Overall, it was higher among individuals born before 1950 and lower in those born thereafter. There was a rapid change in the sanitary conditions and standard of living in Japan after World War Ⅱ, and clean public water systems were introduced in Japan in the 1950s. Therefore, sanitary conditions, such as a full equipment rate of water and sewage, are considered to be important factors for *H. pylori* infection[8].

The prevalence of *H. pylori* in patients with peptic ulcers was significantly higher than that in patients with gastritis, which is consistent with previous reports[16-18]. This observation suggests that *H. pylori* infection is a risk factor for the development of peptic ulcers and gastric cancer, even in Bhutan. In addition, we found 5 gastric

cancer patients among the 372 volunteers. The ASR of gastric cancer in Bhutan was reported to be 24.2/100000 based on the small number in the registry (114 cases) in 2008[4]. This observation supports the high incidence of gastric cancer in Bhutan.

In conclusion, the high incidence of gastric cancer in Bhutan may be attributed to the high prevalence of *H. pylori* infection. Therefore, the eradication therapy of *H. pylori* can contribute to a decrease in *H. pylori*-related diseases, such as peptic ulcers and gastric cancer. However, we should be cautious regarding eradication therapy in Bhutan. Even when eradication therapy for *H. pylori* has succeeded, the infection frequently recurs in patients in developing countries, where there is a high prevalence of *H. pylori* infection[19]. Such repeat infections are either due to a recurrence of the original infection or reinfection with a new strain. Improving the sanitary conditions to decrease the prevalence of *H. pylori* in Bhutan is important.

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COMMENTS

Background

Bhutan is a small landlocked country in South Asia and the prevalence of *Helicobacter pylori* (*H. pylori*) infection in Bhutan has not been investigated.

Research frontiers

The prevalence of *H. pylori* in Bhutan was 73.4% and the high incidence of gastric cancer and peptic ulcer disease in Bhutan may be attributed to the high prevalence of *H. pylori* infection.

Innovations and breakthroughs

This is the first study exploring the extremely high prevalence of *H. pylori* infection in Bhutan. Many tests for *H. pylori* detection such as serological test rapid urease test, culture, histology and immunohistochemistry were performed and analyzed.

Applications

The study results suggest that high incidence of gastric cancer and peptic ulcer disease in Bhutan may be attributed to the high prevalence of *H. pylori* infection. Therefore, *H. pylori* eradication therapy can contribute to reduce these *H. pylori*-related diseases.

Peer review

This is a study in which authors analyzed the prevalence of *H. pylori* infection in different cities, age group and each disease. The results are very interesting and suggest that *H. pylori* eradication and improve of sanitation should be considered to reduce *H. pylori* infection and related diseases in Bhutan.

REFERENCES

1 **Peek RM**, Blaser MJ. Helicobacter pylori and gastrointestinal tract adenocarcinomas. *Nat Rev Cancer* 2002; **2**: 28-37 [PMID: 11902583 DOI: 10.1038/nrc703]
2 **Suerbaum S**, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002; **347**: 1175-1186 [PMID: 12374879 DOI: 10.1056/NEJMra020542]
3 **Fox JG**, Wang TC. Inflammation, atrophy, and gastric cancer. *J Clin Invest* 2007; **117**: 60-69 [PMID: 17200707 DOI: 10.1172/JCI30111]
4 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]
5 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789 [PMID: 11556297 DOI: 10.1056/NEJMoa001999]
6 **Parsonnet J**, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325**: 1127-1131 [PMID: 1891020]
7 **Miwa H**, Go MF, Sato N. H. pylori and gastric cancer: the Asian enigma. *Am J Gastroenterol* 2002; **97**: 1106-1112 [PMID: 12014714 DOI: 10.1111/j.1572-0241.2002.05663.x]
8 **Goh KL**, Chan WK, Shiota S, Yamaoka Y. Epidemiology of Helicobacter pylori infection and public health implications. *Helicobacter* 2011; **16** (Suppl 1): 1-9 [PMID: 21896079 DOI: 10.1111/j.1523-5378.2011.00874.x]
9 **Azevedo NF**, Huntington J, Goodman KJ. The epidemiology of Helicobacter pylori and public health implications. *Helicobacter* 2009; **14** (Suppl 1): 1-7 [PMID: 19712161 DOI: 10.1111/j.1523-5378.2009.00703.x]
10 **Tonkic A**, Tonkic M, Lehours P, Mégraud F. Epidemiology and diagnosis of Helicobacter pylori infection. *Helicobacter* 2012; **17** (Suppl 1): 1-8 [PMID: 22958148 DOI: 10.1111/j.1523-5378.2012.00975.x]
11 **Dixon MF**, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996; **20**: 1161-1181 [PMID: 8827022]
12 **Uchida T**, Kanada R, Tsukamoto Y, Hijiya N, Matsuura K, Yano S, Yokoyama S, Kishida T, Kodama M, Murakami K, Fujioka T, Moriyama M. Immunohistochemical diagnosis of the cagA-gene genotype of Helicobacter pylori with anti-East Asian CagA-specific antibody. *Cancer Sci* 2007; **98**: 521-528 [PMID: 17284255]
13 **Frenck RW**, Clemens J. Helicobacter in the developing world. *Microbes Infect* 2003; **5**: 705-713 [PMID: 12814771]
14 **Mégraud F**, Lehours P. Helicobacter pylori detection and antimicrobial susceptibility testing. *Clin Microbiol Rev* 2007; **20**: 280-322 [PMID: 17428887 DOI: 10.1128/CMR.00033-06]
15 **Asaka M**, Kimura T, Kudo M, Takeda H, Mitani S, Miyazaki T, Miki K, Graham DY. Relationship of Helicobacter pylori to serum pepsinogens in an asymptomatic Japanese population. *Gastroenterology* 1992; **102**: 760-766 [PMID: 1537513]
16 **Malfertheiner P**, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. *Gut* 2012; **61**: 646-664 [PMID: 22491499 DOI: 10.1136/gutjnl-2012-302084]
17 **Huang JQ**, Sridhar S, Hunt RH. Role of Helicobacter pylori infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. *Lancet* 2002; **359**: 14-22 [PMID: 11809181]
18 **Papatheodoridis GV**, Sougioultzis S, Archimandritis AJ. Effects of Helicobacter pylori and nonsteroidal anti-inflammatory drugs on peptic ulcer disease: a systematic review. *Clin Gastroenterol Hepatol* 2006; **4**: 130-142 [PMID: 16469671]
19 **Rimbara E**, Fischbach LA, Graham DY. Optimal therapy for Helicobacter pylori infections. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 79-88 [PMID: 21293508 DOI: 10.1038/nrgastro.2010.210]

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BRIEF ARTICLE

Prognostic value of preoperative mean corpuscular volume in esophageal squamous cell carcinoma

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Abstract

AIM: To evaluate whether preoperative mean corpuscular volume (MCV) is a prognostic indicator in patients with resectable esophageal squamous cell carcinoma (ESCC).

METHODS: A total of 298 consecutive, prospectively enrolled patients with histologically diagnosed ESCC who underwent surgery with curative intent from 2001 to 2011 were retrospectively evaluated. Patients were excluded if they had previous malignant disease, distant metastasis at the time of primary treatment, a history of neoadjuvant treatment, had undergone non-radical resection, or had died of a non-tumor-associated cause. Survival status was verified in September 2011. Pathological staging was performed based on the 2010 American Joint Committee on Cancer criteria. Preoperative MCV was obtained from blood counts performed routinely within 7 d prior to surgery. Receiver operating characteristic (ROC) curve analysis was used to determine a cutoff for preoperative MCV.

RESULTS: The 298 patients consisted of 230 males and 68 females, with a median follow-up of 30.1 mo. ROC analysis showed an optimal cutoff for preoperative MCV of 95.6 fl. Fifty-nine patients (19.8%) had high (> 95.6 fl) and 239 (80.2%) had low (≤ 95.6 fl) preoperative MCV. Preoperative MCV was significantly associated with gender (P = 0.003), body mass index (P = 0.017), and preoperative red blood cell count (P < 0.001). The predicted 1-, 3- and 5-year overall survival (OS) rates were 72%, 60% and 52%, respectively. Median OS was significantly longer in patients with low than with high preoperative MCV (27.5 mo *vs* 19.4 mo, P < 0.001). Multivariate analysis showed that advanced pT (P = 0.018) and pN (P < 0.001) stages, upper thoracic location (P = 0.010), lower preoperative albumin concentration (P = 0.002), and high preoperative MCV (P = 0.001) were negative prognostic factors in patients with ESCC. Preoperative MCV also stratified OS in patients with T3, N1-N3, G2-G3 and stage Ⅲ tumors.

CONCLUSION: Preoperative MCV is a prognostic factor in patients with ESCC.

Key words: Preoperative markers; Mean corpuscular volume; Prognosis; Resectable; Esophageal neoplasms

Core tip: Elevated mean corpuscular volume (MCV) has been shown to predict the risk of esophageal squamous cell carcinoma (ESCC). We hypothesized that pretreatment MCV could predict prognosis. In analyzing 298 patients with ESCC, we found that the optimal cut-off

for preoperative MCV was 95.6 fl. Multivariate analysis showed that high (> 95.6 fl) preoperative MCV was a negative prognostic factor, along with advanced stage, upper thoracic location and lower preoperative albumin, in patients with ESCC. Median overall survival was significantly longer in patients with low (≤ 95.6 fl) than high preoperative MCV (27.5 mo *vs* 19.5 mo, $P < 0.001$).

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INTRODUCTION

Elevated mean corpuscular volume (MCV) has long been recognized as a biomarker for alcoholic and folate deficient patients[1-3]. Although the nature of the relationship between them remains unclear, recent reports suggested that alcohol-induced folate deficiency can lead to macrocytosis[4]. In addition, MCV was found to be higher in Asian heavy drinkers with inactive aldehyde dehydrogenase-2 (ALDH2)[5,6] and to be a marker for alcohol abuse with inactive heterozygous ALDH2[7,8], suggesting that acetaldehyde is an important contributor to macrocytosis.

Alcohol abuse, and acetaldehyde and folate deficiency, all indicative of poor physical condition, were found to increase susceptibility to esophageal carcinoma[3,9-12], as was macrocytosis[7,13]. In addition, patients with more advanced malignancies frequently present with more severe hematological anomalies[14,15]. These findings led us to hypothesize that pretreatment MCV may predict the prognosis of patients with esophageal carcinoma. We therefore analyzed the association between preoperative MCV and different clinicopathological parameters, as well as the prognostic significance of preoperative MCV in patients with esophageal squamous cell carcinoma (ESCC).

MATERIALS AND METHODS

Patients selection

This study was a retrospective analysis of a prospectively collected database (2001-2011) of 298 consecutive patients with histologically diagnosed ESCC who underwent surgery with curative intent at the Cancer Center of Sun Yat-Sen University, Guangzhou, China. Patients with previous malignancy, distant metastasis, neoadjuvant treatment, non-radical resection (R1/R2), or non-tumor-associated death were excluded. Tumors were pathologically staged using the American Joint Committee on Cancer (2010) staging system. Patients were followed-up in the outpatient clinic every 3-6 mo during the first 3 years and every 12 mo thereafter. Demography and clinical details were extracted from the database (Table 1). Survival status was verified in September 2011 using the best available methods. The study protocol was approved by the medical ethics committee of the Cancer Center of Sun Yat-Sen University, which waived the requirement for informed consent due to the retrospective nature of the study.

Preoperative MCV

Preoperative MCV was determined from preoperative blood counts, performed routinely within 7 d prior to surgery, using a Beckman Counter blood analyzer (version STKS, Beckman Counter Inc., Fullerton, CA, United States). The cut-off for preoperative MCV was defined by receiver operating characteristic (ROC) curve analysis, with the point maximizing the area under the curve being selected.

Statistical analysis

All statistical analysis were performed using the SPSS 19.0 software package (SPSS, Inc., Chicago, IL, United States). The ROC curve was generated and analyzed using MedCalc statistical software package 11.0.1 (MedCalc Software bvba, Mariakerke, Belgium). Correlations between preoperative MCV and clinicopathological characteristics were assessed using the Pearson's χ^2 test. Overall survival (OS) was defined as the interval from the date of surgery to the date of death, or last follow-up. Multivariate Cox regression analysis was performed for all parameters found to be significant by the univariate analysis. Survival was analyzed using the Kaplan-Meier method, and differences between curves were assessed by the Log-Rank test. Statistical significance was defined as a P value < 0.05.

RESULTS

Patient baseline characteristics and preoperative MCV

The 298 patients consisted of 230 males and 68 females, with a median preoperative MCV of 91.0 fl (range: 61.4-112.4 fl). ROC curve analysis showed that the optimal cut-off point maximizing (0.588) was 95.6 fl ($P = 0.0123$), with a sensitivity of 0.867 and a specificity of 0.324. Using this cut-off, 59 patients (19.8%) had high (> 95.6 fl) and 239 (80.2%) had low (≤ 95.6 fl) preoperative MCV. The correlations between preoperative MCV and clinicopathologic parameters are summarized in Table 1. Preoperative MCV was significantly associated with gender ($P = 0.003$), body mass index (BMI) ($P = 0.017$), and preoperative red blood cell (RBC) count ($P < 0.001$; Figure 1).

Survival and preoperative MCV

Over a median follow-up of 30.1 mo, 102 of the 298 patients (34.2%) died of cancer-related causes, whereas the other 196 (65.8%) survived. The median survival time was 25.8 mo (range: 1.6-116.1 mo), and the predicted 1-, 3- and 5-year OS rates after primary surgery were 72%, 60%, and 52% respectively. Median OS was significantly longer in patients with low than high preoperative MCV (27.5 mo *vs* 19.4 mo, $P < 0.001$; Figure 2).

To determine factors independently prognostic of pa-

Table 1 Clinicopathological parameters and preoperative mean corpuscular volume *n* (%)

Characteristics	Case numbers	Preoperative MCV		*P* value Pearson's χ^2 test
		Low	High	
Age, yr (mean ± SE)	58.2 ± 9.2			
≤ 65	231	184 (79.7)	47 (20.3)	
> 65	67	55 (82.1)	12 (17.9)	0.660
Gender				
Male	230	176 (76.5)	54 (23.5)	
Female	68	63 (92.6)	5 (7.4)	0.003
BMI, kg/m^2 (mean ± SE)	22.3 ± 3.2			
≤ 20	65	46 (70.8)	19 (29.2)	
> 20 and ≤ 25	180	144 (80.0)	36 (20.0)	
> 25	53	49 (92.5)	4 (7.5)	0.017
Smoking index	440.1 ± 483.1			
≤ 400	171	141 (82.5)	30 (17.5)	
> 400	127	98 (77.2)	29 (22.8)	0.257
Preoperative RBC, × 10^{12}/L (mean ± SE)	4.5 ± 0.6			
≤ 4.0[1]	56	31 (55.4)	25 (44.6)	
> 4.0	242	208 (86.0)	34 (14.0)	< 0.001
Preoperative albumin, g/L (mean ± SE)	42.9 ± 4.6			
≤ 43[2]	149	115 (77.2)	34 (22.8)	
> 43	149	124 (83.2)	25 (16.8)	0.191
pT status, UICC^{7th} (mean ± SE)				
T1	33	31 (93.9)	2 (6.1)	
T2	52	44 (84.6)	8 (15.4)	
T3	213	164 (77.0)	49 (23.0)	0.051
N0	138	116 (84.1)	22 (15.9)	
N1	89	67 (75.3)	22 (24.7)	
N2	51	42 (82.4)	9 (17.6)	
N3	20	14 (70.0)	6 (30.0)	0.250
Histologic grade				
G1	94	71 (75.5)	23 (24.5)	
G2	156	127 (81.4)	29 (18.6)	
G3	48	41 (85.4)	7 (14.6)	0.324
pTNM stage (UICC^{7th})				
Stage Ⅰ	37	32 (86.5)	5 (13.5)	
Stage Ⅱ	120	100 (83.3)	20 (16.7)	
Stage Ⅲ	141	107 (75.9)	34 (24.1)	0.191
Tumor location				
Upper	48	37 (77.1)	11 (22.9)	
Middle	150	125 (83.3)	25 (16.7)	
Lower	100	77 (77.0)	23 (23.0)	0.393

[1]Normal limit of red blood cell count; [2]Mean value of preoperative hemoglobin. MCV: Mean corpuscular volume; Low: Low preoperative MCV (≤ 95.6 fl); High: High preoperative MCV (> 95.6 fl); BMI: Body mass index; RBC: Red blood cell; UICC: Union for International Cancer Control.

tient survival, we analyzed OS using a Cox proportional hazards model. All parameters found to be potentially significant in univariate analysis were included in a multivariate analysis. We found that pT status ($P = 0.018$), pN status ($P < 0.001$), tumor location ($P = 0.010$), preoperative albumin concentration ($P = 0.002$), and preoperative MCV ($P = 0.001$) were significantly prognostic of survival in this patient cohort (Table 2). When we analyzed the effect of preoperative MCV on OS in patients classified by clinicopathological factors, preoperative MCV was predictive of OS in patients with T3 ($P < 0.001$), N1-N3 ($P < 0.001$), G2-G3 ($P < 0.001$), and stage Ⅲ ($P = 0.001$) tumors (Figure 2 and Table 3).

DISCUSSION

Hematologic parameters have been reported to correlate significantly with prognosis in patients with advanced malignant disease[15-18]. MCV is considered a sensitive indicator of alcohol abuse and folate deficiency[1,2,4,6,19]. Recently, MCV was found to be a biomarker for alcohol abuse accompanied by inactive heterozygous ALDH2, and also allowed for the prediction of ESCC risk[8]. To our knowledge, however, no previous study has assessed the relationship between MCV and the prognosis of patients with ESCC.

Using ROC curve analysis, we found that a cut-off of 95.6 fl was a statistically significant predictor of OS. Moreover, high (> 95.6 fl) MCV was significantly correlated with male gender, lower BMI, and RBC ≤ 4 × 10^{12}/L. Folate deficiency has been shown to inhibit red cell maturation, as well as increasing erythrocyte fragility, resulting in increased hemolysis and lower RBC count, which consequently results in macrocytosis[20]. Lower BMI may accompany poor nutritional status, which was associated with elevated MCV[3,21]. The significant correlation

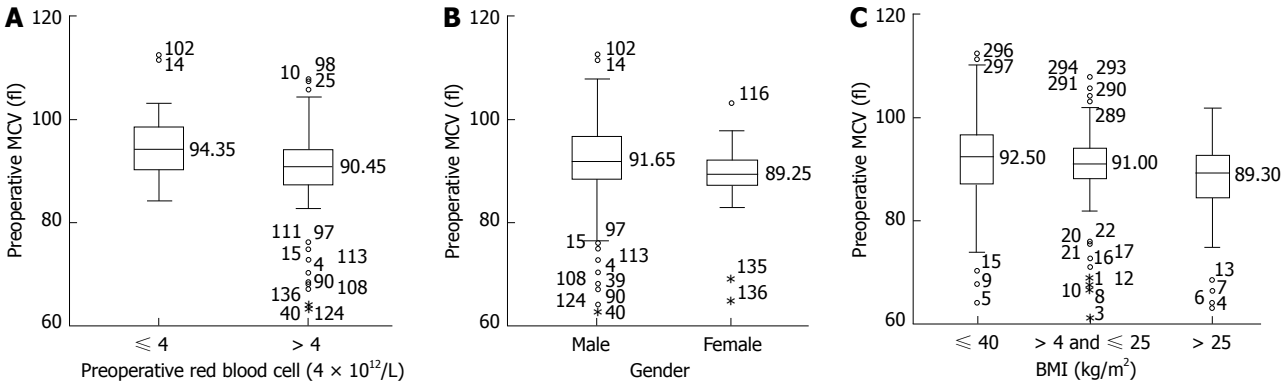

Figure 1 Box plot of preoperative mean corpuscular volume stratified by preoperative red blood cell count (A), gender (B) and body mass index (C). MCV: Mean corpuscular volume; BMI: Body mass index.

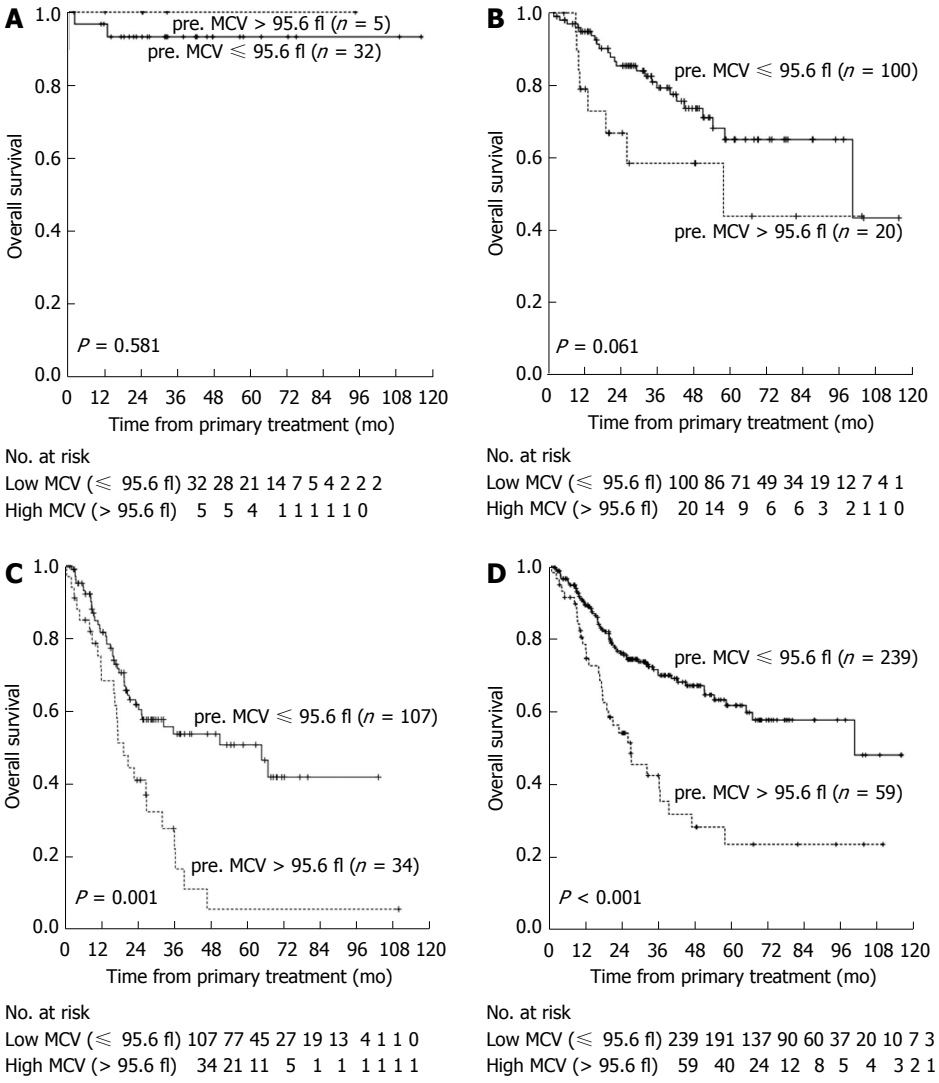

Figure 2 Kaplan-Meier estimates of the probability of overall survival according to preoperative mean corpuscular volume in stage Ⅰ cohort (A), stage Ⅱ cohort (B), stage Ⅲ cohort (C), and all cohorts (D). pre. MCV: Preoperative mean corpuscular volume.

between high MCV and male gender may be related to the association between macrocytosis and alcohol abuse, since overdrinking is much more frequent in males than in females[6-8]. Furthermore, MCV tended to be associated with pT status ($P = 0.051$), consistent with findings showing that preoperative MCV may provide a complementary advantage in assessing tumor invasiveness[17].

Although TNM stage is the best predictor of survival in cancer patients, OS may differ widely in patients with the same TNM stage tumors who receive the same treatment, suggesting that other, as yet undetermined factors may affect prognosis. Since preoperative hematologic parameters have been predictive of patient prognosis[15,22-25], we performed univariate and multivariate analyses of

Table 2 Univariate and multivariate Cox regression analysis for overall survival

Factors	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P* value[1]	HR (95%CI)	*P* value[1]
Age, yr	1.007 (0.985-1.029)	0.527		
Gender (male *vs* female)	0.744 (0.464-1.196)	0.222		
Smoking index (≤ 400 *vs* > 400)	1.391 (0.940-2.060)	0.099	1.302 (0.874-1.940)	0.194
BMI, kg/m^2 (≤ 20 *vs* > 20; ≤ 25 *vs* > 25)	0.878 (0.638-1.207)	0.422		
Preoperative MCV, fl (≤ 95.6 *vs* > 95.6)	2.495 (1.644-3.787)	< 0.001	2.108 (1.372-3.241)	0.001
Preoperative RBC, × 10^{12}/L (≤ 4 *vs* > 4)	0.685 (0.433-1.082)	0.105	0.835 (0.507-1.350)	0.462
Preoperative albumin, g/L	0.954 (0.919-0.990)	0.012	0.938 (0.900-0.977)	0.002
pT status (pT1 *vs* pT2 and pT3)	1.641 (1.147-2.348)	0.007	1.589 (1.084-2.327)	0.018
pN status (pN0 *vs* pN1, pN2 and pN3)	1.954 (1.603-2.382)	< 0.001	1.957 (1.602-2.392)	< 0.001
Histologic grade (G1 *vs* G2 and G3)	0.961 (0.714-1.293)	0.791		
Tumor location (upper thoracic *vs* middle thoracic and lower thoracic)	0.798 (0.605-1.051)	0.109	0.692 (0.522-0.916)	0.01

[1]Cox proportional hazards model. HR: Hazard ratio; BMI: Body mass index; MCV: Mean corpuscular volume; RBC: Red blood cell.

Table 3 Comparison of prognosis in specified cohort stratified by preoperative mean corpuscular volume

Variable	Case numbers	Overall survival (mo) (mean ± SE)	*P* value Log-Rank test
All cohort			< 0.001
Low	239	33.7 ± 24.7	
High	59	25.8 ± 24.2	
pT status			
T1-T2			0.075
Low	75	39.1 ± 27.1	
High	10	27.6 ± 22.7	
T3			< 0.001
Low	164	31.2 ± 23.2	
High	49	25.4 ± 24.7	
pN status			
N0			0.464
Low	116	40.0 ± 26.0	
High	22	35.5 ± 28.7	
N1-N3			< 0.001
Low	123	27.9 ± 22.0	
High	37	20.0 ± 19.2	
Histologic grade			
G1			0.211
Low	71	32.9 ± 27.6	
High	23	27.4 ± 24.3	
G2-G3			< 0.001
Low	168	34.1 ± 23.4	
High	36	24.8 ± 24.4	
pTNM stage			
Stage Ⅰ			0.581
Low	32	37.9 ± 27.1	
High	5	37.6 ± 32.6	
Stage Ⅱ			0.061
Low	100	39.6 ± 25.4	
High	20	31.1 ± 27.7	
Stage Ⅲ			0.001
Low	107	27.0 ± 21.6	
High	34	20.9 ± 19.8	

Low: Low preoperative mean corpuscular volume (≤ 95.6 fl); High: High preoperative mean corpuscular volume (> 95.6 fl).

factors predictive of OS in patients with ESCC. We found that pathological stage, tumor location, preoperative albumin concentration, and preoperative MCV were prognostic factors in our patient cohort.

We also found that OS was significantly shorter in patients with upper-thoracic cancer than those with middle and lower-thoracic esophageal cancer. A study of 605 patients with ESCC also found that median OS was significantly shorter in patients with upper thoracic cancer than in those with middle and lower thoracic tumors (45.9 mo *vs* 82.2 and 93.8 mo; $P < 0.001$)[26]. Due to their anatomical location, carcinomas of the upper thoracic esophagus often result in early invasion of adjacent structures and extensive lymph node metastasis[27]. The prognostic significance of preoperative albumin concentration may be due to it being a sensitive indicator of nutrition, liver function, and metabolic response to disease; thus patients with lower albumin concentrations may present with poorer physical status, decreasing both their response and tolerance to treatment[28,29]. Similar findings were reported in patients with adenocarcinoma of the gastric cardia[30].

Although we found that preoperative MCV was prognostic in patients with ESCC, there is no evidence that MCV has a direct effect on tumor progression or patient prognosis. MCV, however, is a marker of internal folate concentration. Folate acts to transfer one-carbon moieties, thus playing a central role in DNA synthesis, replication, repair, and methylation[31]. Folate deficiency leads to aberrant DNA methylation, which has been reported to be a predictor of clinical outcome in patients with esophageal cancer[32]. A recent study of 125 ESCC patients who underwent surgical resection showed that median OS was significantly longer in patients with high than with low/moderate folate intake (4.59 years *vs* 3.06 years; $P = 0.007$)[33]. Similar results were reported in patients with advanced gastric cancer who were treated with chemotherapy[34].

Another factor linking MCV with prognosis in ESCC is macrocytosis, which may be an indicator of malnutrition, a negative prognostic factor in various human cancers[21,35,36]. In addition, crystal osmotic pressure was shown to be a major regulator of red cell volume in internal environments[37]. Dysphagia, a frequently observed symptom in patients with advanced esophageal cancer,

restricts intake, thus reducing serum concentrations of electrolytes, glucose, and amino acids. This, in turn, may decrease crystal osmotic pressure, leading to red cell dilation. Our finding, that preoperative MCV was related to pT stage and BMI, was consistent with results suggesting that increased MCV was associated with tumor invasiveness and nutritional status[3,17]. Thus, taken together, these results suggest that preoperative MCV may be a marker reflecting internal folate concentration, nutritional status, and tumor invasiveness, thus comprehensively predicting prognosis in patients with ESCC. MCV assays are also convenient and inexpensive to perform, allowing for wide clinical application and suggesting that they may be crucial in preoperative assessment.

To further evaluate the prognostic significance of preoperative MCV, we performed subgroup analysis in patients with ESCC. We found that MCV resulted in the stratification of OS in patients with T3, N1-N3, G2-G3, and stage Ⅲ tumors, but not in patients with T1-T2, N0, G1, or stage Ⅰ / Ⅱ tumors. These findings, however, may be due to the small sample size of these subgroups. Moreover, the relatively good prognosis in patients with T1/2, N0, G1, and stage Ⅰ / Ⅱ tumors may mask the significance of preoperative MCV.

This study has limitations and potential biases. Due to its retrospective nature, records of alcohol consumption by patients were incomplete and folic acid concentrations were not tested in most patients. Furthermore, we could not determine whether preoperative MCV was a better predictor of OS than conventional prognostic factors. Finally, our small sample size may reflect a selection bias to some extent.

In conclusion, in patients with resectable ESCC, OS was significantly longer in patients with low (≤ 95.6 fl) than high (> 95.6 fl) preoperative MCV. Additional studies, however, are required to validate our results.

COMMENTS

Background

Surgical resection remains the treatment of choice for patients with localized esophageal carcinoma. Routine preoperative blood tests of red blood cells, white blood cells, and platelet counts can help estimate surgical risk. Significant hematologic variations frequently observed in patients with advanced malignant diseases may predict prognosis.

Research frontiers

Elevated mean corpuscular volume (MCV) has long been recognized as a biomarker for alcohol abuse and folate deficiency. In addition, MCV was reported to be higher in Asian heavy drinkers with inactive aldehyde dehydrogenase-2 (ALDH2), and was found to be a marker of alcohol abuse in individuals with inactive heterozygous ALDH2, suggesting that acetaldehyde may be an important contributor to macrocytosis. A recent study showed that macrocytosis was a risk factor for esophageal carcinoma.

Innovations and breakthroughs

The authors observed a correlation between macrocytosis and prognosis in patients with esophageal carcinoma. Overall survival was significantly shorter in patients with elevated MCV than those with lower MCV. Utilizing receiver operating characteristic curve analysis, the authors determined an optimal cut-off point for MCV, which was both reasonable and objective.

Applications

These results suggest that preoperative MCV may be used to predict prognosis in patients with esophageal cancer. Routine blood tests should be performed shortly before surgery in these patients, and those with elevated MCV, especially greater than 95.6 fl, should be carefully evaluated to assess the risks and feasibility of surgery.

Terminology

MCV, representing the mean volume of a single red blood cell, is determined by indirect calculation. Clinically, this parameter is often used in the differential diagnosis of various type of anemia.

Peer review

This is an article on an unusual topic. The value of MCV has been known up to now as risk factor for esophageal carcinoma, but it is not known as prognostic factor.

REFERENCES

1 **Herbert V**, Zalusky R, Davidson Cs. Correlation of folate deficiency with alcoholism and associated macrocytosis, anemia, and liver disease. *Ann Intern Med* 1963; **58**: 977-988 [PMID: 13953905]

2 **Unger KW**, Johnson D. Red blood cell mean corpuscular volume: a potential indicator of alcohol usage in a working population. *Am J Med Sci* 1974; **267**: 281-289 [PMID: 4833835 DOI: 10.1097/00000441-197405000-00004]

3 **World MJ**, Ryle PR, Jones D, Shaw GK, Thomson AD. Differential effect of chronic alcohol intake and poor nutrition on body weight and fat stores. *Alcohol Alcohol* 1984; **19**: 281-290 [PMID: 6532464]

4 **de la Vega MJ**, Santolaria F, González-Reimers E, Alemán MR, Milena A, Martínez-Riera A, González-García C. High prevalence of hyperhomocysteinemia in chronic alcoholism: the importance of the thermolabile form of the enzyme methylenetetrahydrofolate reductase (MTHFR). *Alcohol* 2001; **25**: 59-67 [PMID: 11747974]

5 **Nomura F**, Itoga S, Tamura M, Harada S, Iizuka Y, Nakai T. Biological markers of alcoholism with respect to genotypes of low-Km aldehyde dehydrogenase (ALDH2) in Japanese subjects. *Alcohol Clin Exp Res* 2000; **24**: 30S-33S [PMID: 10803776]

6 **Hashimoto Y**, Nakayama T, Futamura A, Omura M, Nakahara K. Erythrocyte mean cell volume and genetic polymorphism of aldehyde dehydrogenase 2 in alcohol drinkers. *Blood* 2002; **99**: 3487-3488 [PMID: 12001910]

7 **Yokoyama M**, Yokoyama A, Yokoyama T, Hamana G, Funazu K, Kondo S, Yamashita T, Yoshimizu H, Nakamura H. Mean corpuscular volume and the aldehyde dehydrogenase-2 genotype in male Japanese workers. *Alcohol Clin Exp Res* 2003; **27**: 1395-1401 [PMID: 14506399 DOI: 10.1097/01.ALC.0000085589.47243.8D]

8 **Yokoyama A**, Yokoyama T, Muramatsu T, Omori T, Matsushita S, Higuchi S, Maruyama K, Ishii H. Macrocytosis, a new predictor for esophageal squamous cell carcinoma in Japanese alcoholic men. *Carcinogenesis* 2003; **24**: 1773-1778 [PMID: 12949054 DOI: 10.1093/carcin/bgg142]

9 **Homann N**, Stickel F, König IR, Jacobs A, Junghanns K, Benesova M, Schuppan D, Himsel S, Zuber-Jerger I, Hellerbrand C, Ludwig D, Caselmann WH, Seitz HK. Alcohol dehydrogenase 1C*1 allele is a genetic marker for alcohol-associated cancer in heavy drinkers. *Int J Cancer* 2006; **118**: 1998-2002 [PMID: 16287084 DOI: 10.1002/ijc.21583]

10 **Song C**, Xing D, Tan W, Wei Q, Lin D. Methylenetetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population. *Cancer Res* 2001; **61**: 3272-3275 [PMID: 11309278]

11 **Zhang J**, Zotz RB, Li Y, Wang R, Kiel S, Schulz WA, Wen D, Chen Z, Zhang L, Wang S, Gabbert HE, Sarbia M. Methylenetetrahydrofolate reductase C677T polymorphism and predisposition towards esophageal squamous cell carcinoma in a German Caucasian and a northern Chinese population. *J Cancer Res Clin Oncol* 2004; **130**: 574-580 [PMID: 15449187 DOI: 10.1007/s00432-004-0585-4]

12 **Wang LD**, Guo RF, Fan ZM, He X, Gao SS, Guo HQ, Matsuo

K, Yin LM, Li JL. Association of methylenetetrahydrofolate reductase and thymidylate synthase promoter polymorphisms with genetic susceptibility to esophageal and cardia cancer in a Chinese high-risk population. *Dis Esophagus* 2005; **18**: 177-184 [PMID: 16045580]
13 **Yokoyama T**, Yokoyama A, Kato H, Tsujinaka T, Muto M, Omori T, Haneda T, Kumagai Y, Igaki H, Yokoyama M, Watanabe H, Yoshimizu H. Alcohol flushing, alcohol and aldehyde dehydrogenase genotypes, and risk for esophageal squamous cell carcinoma in Japanese men. *Cancer Epidemiol Biomarkers Prev* 2003; **12**: 1227-1233 [PMID: 14652286]
14 **Lee YT**, Van Peenen HJ, Watson FR. Chemical and hematological screening in patients with malignant and non-malignant conditions. *J Surg Oncol* 1980; **15**: 43-51 [PMID: 7421269]
15 **Bhatti I**, Peacock O, Lloyd G, Larvin M, Hall RI. Preoperative hematologic markers as independent predictors of prognosis in resected pancreatic ductal adenocarcinoma: neutrophil-lymphocyte versus platelet-lymphocyte ratio. *Am J Surg* 2010; **200**: 197-203 [PMID: 20122680 DOI: 10.1016/j.amjsurg.2009.08.041]
16 **Banerjee RN**, Narang RM. Haematological changes in malignancy. *Br J Haematol* 1967; **13**: 829-843 [PMID: 6075441 DOI: 10.1111/j.1365-2141.1967.tb08854.x]
17 **Ruka W**, Rutkowski P, Kaminska J, Rysinska A, Steffen J. Alterations of routine blood tests in adult patients with soft tissue sarcomas: relationships to cytokine serum levels and prognostic significance. *Ann Oncol* 2001; **12**: 1423-1432 [PMID: 11762815]
18 **Stock W**, Hoffman R. White blood cells 1: non-malignant disorders. *Lancet* 2000; **355**: 1351-1357 [PMID: 10776761 DOI: 10.1016/S0140-6736(00)02125-5]
19 **Chalmers DM**, Levi AJ, Chanarin I, North WR, Meade TW. Mean cell volume in a working population: the effects of age, smoking, alcohol and oral contraception. *Br J Haematol* 1979; **43**: 631-636 [PMID: 526445 DOI: 10.1111/j.1365-2141.1979.tb03796.x]
20 **Weiser G**, Kohn C, Vachon A. Erythrocyte volume distribution analysis and hematologic changes in two horses with immune-mediated hemolytic anemia. *Vet Pathol* 1983; **20**: 424-433 [PMID: 6623846 DOI: 10.1177/030098588302000405]
21 **Wu A**, Chanarin I, Levi AJ. Macrocytosis of chronic alcoholism. *Lancet* 1974; **1**: 829-831 [PMID: 4132788]
22 **Bruckner HW**, Lavin PT, Plaxe SC, Storch JA, Livstone EM. Absolute granulocyte, lymphocyte, and moncyte counts. Useful determinants of prognosis for patients with metastatic cancer of the stomach. *JAMA* 1982; **247**: 1004-1006 [PMID: 7035703 DOI: 10.1001/jama.1982.03320320040027]
23 **Riesco A**. Five-year cancer cure: relation to total amount of peripheral lymphocytes and neutrophils. *Cancer* 1970; **25**: 135-140 [PMID: 5410303]
24 **Sasaki A**, Iwashita Y, Shibata K, Matsumoto T, Ohta M, Kitano S. Prognostic value of preoperative peripheral blood monocyte count in patients with hepatocellular carcinoma. *Surgery* 2006; **139**: 755-764 [PMID: 16782430 DOI: 10.1016/j.surg.2005.10.009]
25 **Sasaki A**, Kai S, Endo Y, Iwaki K, Uchida H, Tominaga M, Okunaga R, Shibata K, Ohta M, Kitano S. Prognostic value of preoperative peripheral blood monocyte count in patients with colorectal liver metastasis after liver resection. *J Gastrointest Surg* 2007; **11**: 596-602 [PMID: 17468918 DOI: 10.1007/s11605-007-0140-0]
26 **Li H**, Zhang Q, Xu L, Chen Y, Wei Y, Zhou G. Factors predictive of prognosis after esophagectomy for squamous cell cancer. *J Thorac Cardiovasc Surg* 2009; **137**: 55-59 [PMID: 19154903 DOI: 10.1016/j.jtcvs.2008.05.024]
27 **Wang HW**, Kuo KT, Wu YC, Huang BS, Hsu WH, Huang MH, Wang LS. Surgical results of upper thoracic esophageal carcinoma. *J Chin Med Assoc* 2004; **67**: 447-457 [PMID: 15617305]
28 **Haupt W**, Holzheimer RG, Riese J, Klein P, Hohenberger W. Association of low preoperative serum albumin concentrations and the acute phase response. *Eur J Surg* 1999; **165**: 307-313 [PMID: 10365830 DOI: 10.1080/110241599750006820]
29 **Danielsen PL**, Agren MS, Jorgensen LN. Platelet-rich fibrin versus albumin in surgical wound repair: a randomized trial with paired design. *Ann Surg* 2010; **251**: 825-831 [PMID: 20395860 DOI: 10.1097/SLA.0b013e3181d3548c]
30 **Lien YC**, Hsieh CC, Wu YC, Hsu HS, Hsu WH, Wang LS, Huang MH, Huang BS. Preoperative serum albumin level is a prognostic indicator for adenocarcinoma of the gastric cardia. *J Gastrointest Surg* 2004; **8**: 1041-1048 [PMID: 15585392 DOI: 10.1016/j.gassur.2004.09.033]
31 **Kim YI**. Will mandatory folic acid fortification prevent or promote cancer? *Am J Clin Nutr* 2004; **80**: 1123-1128 [PMID: 15531657]
32 **Sato F**, Meltzer SJ. CpG island hypermethylation in progression of esophageal and gastric cancer. *Cancer* 2006; **106**: 483-493 [PMID: 16362978 DOI: 10.1002/cncr.21657]
33 **Lu C**, Xie H, Wang F, Shen H, Wang J. Diet folate, DNA methylation and genetic polymorphisms of MTHFR C677T in association with the prognosis of esophageal squamous cell carcinoma. *BMC Cancer* 2011; **11**: 91 [PMID: 21375764 DOI: 10.1186/1471-2407-11-91]
34 **Shitara K**, Muro K, Ito S, Sawaki A, Tajika M, Kawai H, Yokota T, Takahari D, Shibata T, Ura T, Ito H, Hosono S, Kawase T, Watanabe M, Tajima K, Yatabe Y, Tanaka H, Matsuo K. Folate intake along with genetic polymorphisms in methylenetetrahydrofolate reductase and thymidylate synthase in patients with advanced gastric cancer. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 1311-1319 [PMID: 20447923]
35 **Oñate-Ocaña LF**, Aiello-Crocifoglio V, Gallardo-Rincón D, Herrera-Goepfert R, Brom-Valladares R, Carrillo JF, Cervera E, Mohar-Betancourt A. Serum albumin as a significant prognostic factor for patients with gastric carcinoma. *Ann Surg Oncol* 2007; **14**: 381-389 [PMID: 17160496 DOI: 10.1245/s10434-006-9093-x]
36 **Espinosa E**, Feliu J, Zamora P, González Barón M, Sánchez JJ, Ordón ez A, Espinosa J. Serum albumin and other prognostic factors related to response and survival in patients with advanced non-small cell lung cancer. *Lung Cancer* 1995; **12**: 67-76 [PMID: 7600032]
37 **Porath-Furedi A**. The mutual effect of hydrogen ion concentration and osmotic pressure on the shape of the human erythrocyte as determined by light scattering and by electronic cell volume measurement. *Cytometry* 1983; **4**: 263-267 [PMID: 6661992 DOI: 10.1002/cyto.990040312]

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BRIEF ARTICLE

Increased CD163 expression is associated with acute-on-chronic hepatitis B liver failure

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Abstract

AIM: To assess CD163 expression in plasma and peripheral blood and analyze its association with disease in acute-on-chronic hepatitis B liver failure (ACHBLF) patients.

METHODS: A retrospective study was conducted from January 1, 2011 to January 1, 2012. Forty patients with ACHBLF (mean age 44.48 ± 12.28 years, range 18-69 years), 40 patients with chronic hepatitis B (CHB) (mean age 39.45 ± 12.22 years, range 21-57 years) and 20 age- and sex-matched healthy controls (mean age 38.35 ± 11.97 years, range 28-60 years) were included in this study. Flow cytometry was used to analyze the frequency of CD163+ peripheral blood mononuclear cells (PBMCs) and surface protein expression of CD163. Real-time transcription-polymerase chain reaction was performed to assess relative CD163 mRNA levels in PBMCs. Plasma soluble CD163 (sCD163) levels were measured by enzyme-linked immunosorbent assay. Clinical variables were also recorded. Comparisons between groups were analyzed by Kruskal-Wallis *H* test and Mann-Whitney *U* test. Statistical analyses were performed using SPSS 15.0 software and a P value < 0.05 was considered statistically significant.

RESULTS: Flow cytometry showed that the population of CD163+ PBMCs was significantly greater in ACHBLF patients than in CHB patients and healthy controls (47.9645% ± 17.1542%, 32.0975% ± 11.0215% *vs* 17.9460% ± 6.3618%, $P < 0.0001$). However, there were no significant differences in mean fluorescence intensity of CD163+ PBMCs within the three groups (27.4975 ± 11.3731, 25.8140 ± 10.0649 *vs* 20.5050 ± 6.2437, $P = 0.0514$). CD163 mRNA expression in ACHBLF patients was significantly increased compared with CHB patients and healthy controls ($1.41 \times 10^{-2} \pm 2.18 \times 10^{-2}$, $5.10 \times 10^{-3} \pm 3.61 \times 10^{-3}$ *vs* $37.0 \times 10^{-4} \pm 3.55 \times 10^{-4}$, $P = 0.02$). Plasma sCD163 levels in patients with ACHBLF were significantly increased compared with CHB patients and healthy controls (4706.2175 ± 1681.1096 ng/mL, 1089.7160 ± 736.8395 ng/mL *vs* 435.9562 ± 440.8329 ng/mL, $P < 0.0001$). In ACHBLF patients, plasma sCD163 levels were significantly positively associated with model for end-stage liver disease scores ($r = 0.5075$, $P = 0.008$), hepatitis B virus-DNA ($r = 0.6827$, $P < 0.0001$), and negatively associated with prothrombin activity ($r = -0.3348$, $P = 0.0347$), but had no correlation with total bilirubin ($r = 0.2551$, $P = 0.1122$). Furthermore, sCD163 was obviously elevated in non-surviving patients compared with surviving patients with ACHBLF (5344.9080 ± 1589.5199 ng/mL *vs* 3641.7333 ± 1264.5228 ng/mL, $P = 0.0321$).

CONCLUSION: CD163 and sCD163 may be related to disease severity and prognosis in ACHBLF patients.

Key words: Acute-on-chronic hepatitis B liver failure; Model for end-stage liver disease; CD163; Soluble CD163; Real-time transcription-polymerase chain reaction

Core tip: This study included three groups, acute-on-chronic hepatitis B liver failure (ACHBLF) patients, chronic hepatitis B (CHB) patients and healthy controls. Flow cytometry was used to analyze the frequency of CD163+ peripheral blood mononuclear cells (PBMCs) and surface protein expression of CD163. Real-time transcription-polymerase chain reaction was performed to assess relative CD163 mRNA levels in PBMCs. The population of CD163+ PBMCs was significantly larger in ACHBLF patients than in CHB patients and healthy controls. CD163 mRNA expression in ACHBLF patients was significantly increased compared with healthy controls. Plasma soluble CD163 (sCD163) levels were markedly increased and correlated with disease severity and prognosis in ACHBLF patients. CD163 and sCD163 may be useful biomarkers for ACHBLF.

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INTRODUCTION

Hepatitis B virus (HBV) infection is a major health problem worldwide. It is thought to be one of the main causes of liver-related chronic and acute diseases[1]. With severe acute exacerbation of the disease, some chronic hepatitis B (CHB) patients may progress to liver failure. We define this progression as acute-on-chronic hepatitis B liver failure (ACHBLF), and constitutes about 70% of all acute-on-chronic liver failure in areas with a high incidence of hepatitis B[2]. ACHBLF has an extremely poor prognosis due to a lack of understanding of its pathogenesis. Currently, clinical observations have shown that ACHBLF may be related to strong immune responses. Therefore, it is important to fully understand the course of the immune pathogenesis of ACHBLF. If we can identify efficient markers which predict disease progression, this may be of great help in the treatment of ACHBLF[3] .

Innate immune cells such as macrophages can be activated by acute and chronic inflammation and produce various cytokines. These immunological cytokines induce and cause liver tissue injury. Activated macrophages play important roles in the production of these immune cytokines. Monocytes and macrophages can be activated into M1 and M2 subpopulations in response to different environmental signals which are mainly derived from inflammatory diseases. Those of the M2 subpopulation inhibit the inflammatory state[4-6].

CD163 is a member of a scavenger receptor family and is expressed mainly on activated macrophages. It is a specific M2 macrophage marker. Soluble CD163 (sCD163) emerges from the shedding of CD163 from the cell surface in the plasma[6-9]. It is now evident that sCD163 and CD163 are very useful as biomarkers of macrophage activation in various inflammatory diseases. It is strongly indicated that CD163 and sCD163 may be involved in the pathogenesis of liver failure. However, the exact expression of CD163 and sCD163 in ACHBLF patients has not been fully elucidated[10,11].

This study aimed to evaluate peripheral blood CD163 and plasma sCD163 expression in macrophages from patients with ACHBLF.

MATERIALS AND METHODS

Patients

Forty patients with ACHBLF, 40 patients with CHB and 20 healthy controls from Qilu Hospital of Shandong University, were included in this retrospectively study.

Blood samples collected from January 2011 to January 2012 at the Department of Hepatology, Qilu Hospital of Shandong University, were separated into plasma and stored at -70 ℃ until use.

Patients with CHB had more than twice the normal alanine aminotransferase level. All groups were matched for sex and age. ACHBLF patients had a history of CHB, with plasma total bilirubin (TBIL $\geqslant$ 85 μmol/L), prothrombin activity (PTA) < 40%, and complications such as hepatic encephalopathy (no less than grade Ⅱ), ascites or hepato-renal syndrome. According to the Asian Pacific Association for the Study of the Liver guideline, all ACHBLF patients received inpatient treatment. We excluded patients who underwent liver transplantation, had hepatocellular carcinoma or other metastatic liver tumors or who had received immunotherapy or anti-viral treatment within 6 mo. Patients with a history of alcohol abuse, intravenous drug abuse, pregnancy, concomitant chronic hepatitis C, human immune deficiency virus infection or autoimmune hepatitis were also excluded. Hepatitis B surface antigen in healthy controls (n = 20) (age-, sex- and race-matched) was negative. Experiments and procedures were conducted with the guidance of the Helsinki Declaration of 1975[12]. The study was approved by the local Ethical Committee of Qilu Hospital of Shandong University. Prior to the collection of blood, informed consent was obtained from each patient. The characteristics of the enrolled subjects are summarized in Table 1.

RNA extraction and real-time reverse-transcriptase polymerase chain reaction

Six milliliters of peripheral venous blood were collected from each subject and Ficoll-Paque Plus (GE Healthcare, Uppsala, Sweden) was used for isolation of PBMCs. Total RNA in PBMCs was extracted using Trizol (Invitrogen, Carlsbad, CA, United States) according to the manufacturer's instructions. Two micrograms of total RNA were converted into cDNAs using the RevertAid™ First

Table 1 Baseline characteristics of the subjects enrolled in the present study

Patients	ACHBLF	CHB	Healthy control
Cases	40	40	20
Age, yr	44.48 ± 12.28	39.45 ± 12.22	38.35 ± 11.97
Sex (male/female)	23/17	26/14	12/8
HBeAg (+)/HBeAg (-)	19/21	28/12	NA
PTA, %	45.36 ± 25.14	98.70 ± 17.56	108.00 ± 13.56
TBIL, μmol/L	322.08 ± 166.00	33.39 ± 66.35	18.65 ± 6.55
HBV DNA, log10copies/mL	322.08 ± 166.00	3.20 ± 1.44	NA
Encephalopathy grade Ⅲ/Ⅳ	12.12%	0%	0%
Ascites	30.30%	0%	0%
Mortality	62.5%	0%	0%

ACHBLF: Acute-on-chronic hepatitis B liver failure; CHB: Chronic hepatitis B; HBeAg: Hepatitis B e antigen; PTA: Prothrombin activity; TBIL: Total bilirubin; HBV: Hepatitis B virus; NA: Not available.

Strand cDNA Synthesis Kit (Fermentas, Lithuania) and real-time polymerase chain reaction (RT-PCR) was carried out on a Lightcycler (Roche Diagnostics, Germany). RT-PCR amplification mixtures (20 μL) contained 75 ng template cDNA, 10 μL 2 9 SYBR@ Premix Ex Taq™ (Takara, Japan) and 200 nmol/L forward and reverse primer. The real-time PCR reaction was performed as follows: the initial step was 95 ℃ for 30 s, followed by 40 cycles at 95 ℃ for 5 s, 60 ℃ for 30 s and 72 ℃ for 30 s. β-actin was used as the endogenous control for the normalization of RNA quantity and quality differences in all samples.

The primers were as follows: CD163 forward 5'-TCTGGCTTGACAGCGTTTC-3'; CD163 reverse 5'-TGTGTTTGTTGCCTGGATT-3'; β-actin forward 5'-CGGGAAATCGTGCGTGACATT-3'; β-actin reverse 5'-GGAGTTGAAGGTAGTTTCGTGG-3' (Fermentas, Lithuania). Gene specific amplifications were demonstrated with melting curve data and gel-migration analyses.

Flow cytometric analysis

Freshly drawn peripheral whole blood samples of 200 μL were stained with isotype-matched control antibody or a relevant antibody (CD14, CD163) for 30 min at room temperature in the dark. Anti-human CD163 PE, anti-human CD14 APC and isotype-matched control antibody were purchased from eBioscience (San Diego, CA, United States). Following incubation, erythrocytes were lysed with RBC lysis buffer (Whole Blood Lysing Kit, R and D Systems, United States). Finally, the cells were washed three times, resuspended in 500 μL of PBS containing 0.5% formaldehyde, and analyzed using a FACS Calibur (BD Bioscience, PharMingen). The samples were analyzed with a Becton-Dickinson FACS Calibur machine. Separate gates were established for the macrophages. The amount of CD163 in peripheral blood was assessed using flow cytometry (Coulter counter)[13-15] (Figure 1).

Determination of plasma levels of sCD163

SCD163 was determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (R and D, Minneapolis, MN, United States). All samples were analyzed in duplicate following the manufacturer's protocol. The sensitivity of the assay was 0.1 ng/mL, and the intra-assay and inter-assay coefficients of variation were less than 3% and 6%, respectively.

Clinical and laboratory parameters

Measurement of liver function tests: TBIL and hematological tests including PTA were performed using standard methods in a clinical setting. Hepatitis B markers were tested using a commercially available radioimmunoassay (Abbott). The level of HBV DNA was quantified using a DNA assay (sensitivity > 500 copies/mL). Model for end-stage liver disease (MELD) scores were calculated according to the Malinchoc formula: $r = 9.57 \times \log_e$ [creatinine (mg/dL)] + 3.78 × $\log_e$ [bilirubin (mg/dL)] + 11.2 × $\log_e$ (INR) + 6.43 × (etiology: 0 if cholestatic or alcoholic, 1 otherwise)[16,17].

Statistical analysis

Statistical analysis were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, United States). Comparisons between groups were analyzed by Kruskal-Wallis *H* test and Mann-Whitney *U* test. The Spearman rank correlation test was used for correlation analysis. All statistical analysis were two-sided, and a P value < 0.05 was considered statistically significant.

RESULTS

Frequency of circulating CD163+ PBMCs and mean fluorescence intensity

We determined the frequency of CD163+ PBMCs using flow cytometry and found that in ACHBLF patients, the frequency was markedly higher than that in the healthy control group and CHB patients, respectively (Figure 2A). There was a significant difference in the frequency of CD163+ PBMCs within the three groups (47.9645% ± 17.1542%, 32.0975% ± 11.0215% *vs* 17.9460% ± 6.3618%, $P < 0.0001$). We also evaluated the average mean fluorescence intensity (MFI) of CD163+ PBMCs using flow cytometric analysis. However, there were no significant differences in MFI in CD163 + PBMCs within the three groups (27.4975 ± 11.3731, 25.8140 ± 10.0649 *vs* 20.5050 ± 6.2437, $P = 0.0514$) (Figure 2B).

Plasma levels of sCD163 in ACHBLF patients

We evaluated the plasma levels of sCD163 by ELISA. The results showed that the levels of sCD163 in ACHBLF patients were markedly higher than those in CHB patients and the healthy control group. Significant differences in plasma sCD163 were found within ACHBLF patients, CHB patients and healthy controls (4706.2175 ± 1681.1096 ng/mL, 1089.7160 ± 736.8395 ng/mL *vs* 435.9562 ± 440.8329 ng/mL, $P < 0.0001$) (Figure 2C).

Increased mRNA expression of CD163 in PBMCs from ACHBLF patients

We also determined the mRNA level of CD163 in PBMCs

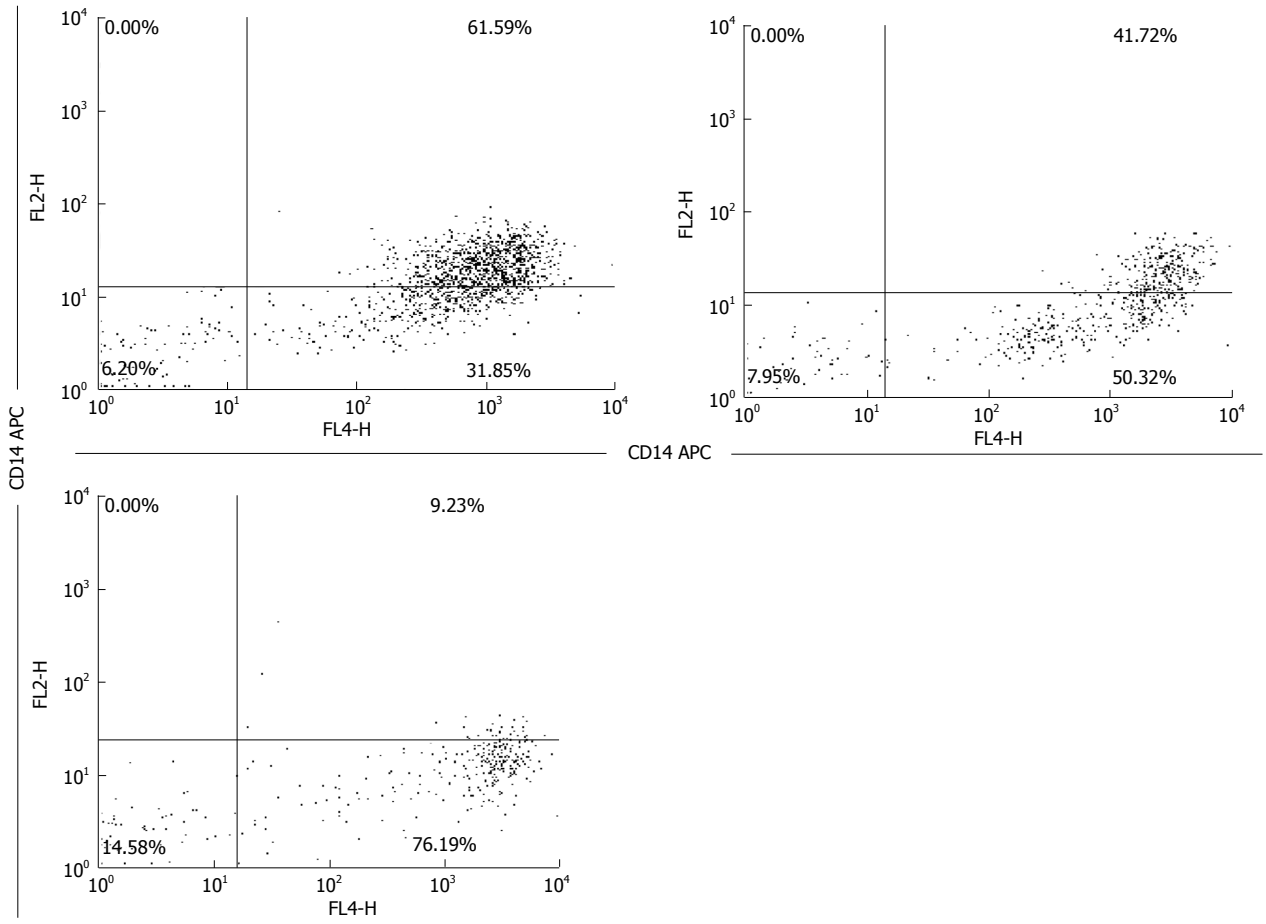

Figure 1 Percentage of CD163+ peripheral blood mononuclear cells. The representative results of CD163+ peripheral blood mononuclear cells are shown. ACHBLF: Acute-on-chronic hepatitis B liver failure; CHB: Chronic hepatitis B.

using RT-PCR. No significant differences in the mRNA level of CD163 were found within the three groups ($1.41 \times 10^{-2} \pm 2.18 \times 10^{-2}$, $5.10 \times 10^{-3} \pm 3.61 \times 10^{-3}$ *vs* $37.0 \times 10^{-4} \pm 3.55 \times 10^{-4}$, $P = 0.02$). The mRNA levels of CD163 in ACHBLF patients and CHB patients were significantly higher than those in healthy controls. No significant difference in the mRNA level of CD163 was observed in ACHBLF and CHB patients ($P > 0.05$) (Figure 2D).

Increased plasma sCD163 levels in patients with ACHBLF

To determine whether the increase in plasma levels of sCD163 correlated with liver injury, we analyzed the plasma levels of sCD163 in ACHBLF patients with clinical and laboratory parameters which indicated liver function or DNA replication in ACHBLF. The results showed that plasma levels of sCD163 were positively correlated with MELD score ($r = 0.5075$, $P = 0.008$), plasma HBV-DNA levels ($r = 0.6827$, $P < 0.0001$) and negatively correlated with PTA ($r = -0.3348$, $P = 0.0347$), but had no correlation with TBIL ($r = 0.2551$, $P = 0.1122$) (Figure 3A-D).

Plasma levels of sCD163 influence disease progression in ACHBLF patients

ACHBLF patients were grouped into survivors and non-survivors after 3 mo of follow up. The plasma levels of sCD163 were increased in non-survivors compared with survivors (5344.9080 ± 1589.5199 ng/mL *vs* 3641.7333 ± 1264.5228 ng/mL, $P = 0.0321$) (Figure 3E).

DISCUSSION

Although sCD163 is thought to play an important role in the pathogenesis of liver failure, this is the first study on the frequency of CD163+ PBMCs and CD163 mRNA level in ACHBLF patients[18,19]. Increased plasma sCD163 levels were correlated with disease severity in ACHBLF patients. These results strongly suggest that the role of CD163 in the immune response might affect the prognosis of ACHBLF.

CD163 is a scavenger receptor, and its expression has been proved to be associated with inhibition of inflammation. Furthermore, during the innate immune response, CD163 plays an important role in the host defense against inflammation[5,20,21]. The soluble form of CD163 is also a biomarker associated with inflammation[18]. In the present study, we first found that the frequency of CD163+ PBMCs and CD163 mRNA expression in PBMCs were dramatically increased in ACHBLF patients compared with healthy controls. These results could be interpreted together with previous reports to suggest that CD163 might

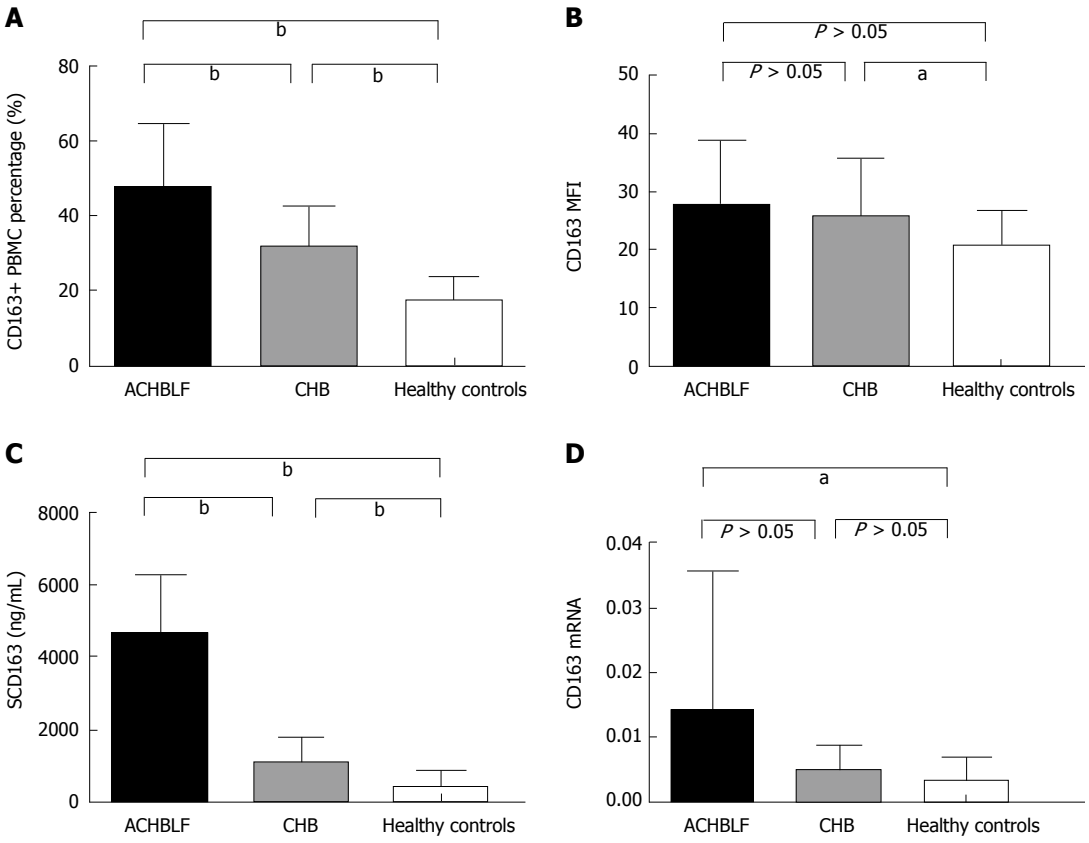

Figure 2 Acute-on-chronic hepatitis B liver failure (*n* = 40), chronic hepatitis B (*n* = 40) and healthy controls (*n* = 20). A: The frequency of CD163+ peripheral blood mononuclear cells (PBMCs) in acute-on-chronic hepatitis B liver failure (ACHBLF) patients was significantly higher than that in chronic hepatitis B (CHB) patients and healthy controls ($P < 0.01$); B: There were no significant differences in mean fluorescence intensity (MFI) of CD163+ PBMCs within the three groups ($P > 0.05$); C: The plasma levels of soluble CD163 (sCD163) in ACHBLF patients were significantly higher than those in CHB patients and healthy controls ($P < 0.01$); D: The mRNA levels of CD163 in ACHBLF patients and CHB patients were significantly higher than those in healthy controls ($P < 0.01$). Significant differences were calculated using the Kruskal-Wallis *H* test and Mann-Whitney *U* test ($^aP < 0.05$, $^bP < 0.01$ between the two groups).

have a key position in the immunolesion of ACHBLF.

There were no significant differences in MFI of CD163+ PBMCs within the three groups. This result is very interesting. From previous literature, which mainly included randomly selected patients, CD163 was inversely correlated with sCD163 plasma levels[22]. Lipopolysaccharide and other pro-inflammatory cytokines induce shedding of CD163 from the surface of isolated monocytes. During infection, the expression of monocyte surface CD163 decreases. In contrast, some studies have shown that with chronic and acute inflammation, the expression of surface CD163 increased. Through CD163 shedding, decreased surface CD163 was followed by recovery and induction of surface CD163 to higher levels[23-25]. We speculate that surface CD163 expression might vary according to different inflammatory states in patients and different stages of inflammation. More research is needed to confirm this.

In this study, we observed that peripheral mRNA levels of CD163 in ACHBLF patients were high and there was a significant difference between the three groups, however, mRNA levels of CD163 in ACHBLF patients were not significantly different to those in CHB patients. During inflammation, we speculate that plasma sCD163 levels might increase dramatically, but mRNA levels increase slowly[26-28].

In the present study, we found that plasma sCD163 levels were significantly increased in patients with ACHBLF compared to CHB patients and healthy controls. Plasma sCD163 levels were positively associated with HBV DNA levels, MELD scores and were negatively correlated with PTA. In addition, there were no associations with TBIL. It was demonstrated that plasma sCD163 correlated with the severity of ACHBLF[29-31].

The MELD scoring system is widely accepted and is used to predict prognosis in patients with liver failure. We chose the MELD scoring system to evaluate the prognosis of ACHBLF patients over a three month period. The results strongly indicated the potential role of plasma sCD163 levels in forecasting the prognosis of ACHBLF patients. Furthermore, plasma sCD163 levels were obviously elevated in ACHBLF patients who did not survive compared with surviving ACHBLF patients[16,17].

We found significant associations between plasma sCD163 levels and HBV DNA in ACHBLF patients. These results indicate that HBV may contribute to the aggravated macrophage immune response besides activation of HBV[32,33]. HBV might play a key role in promot-

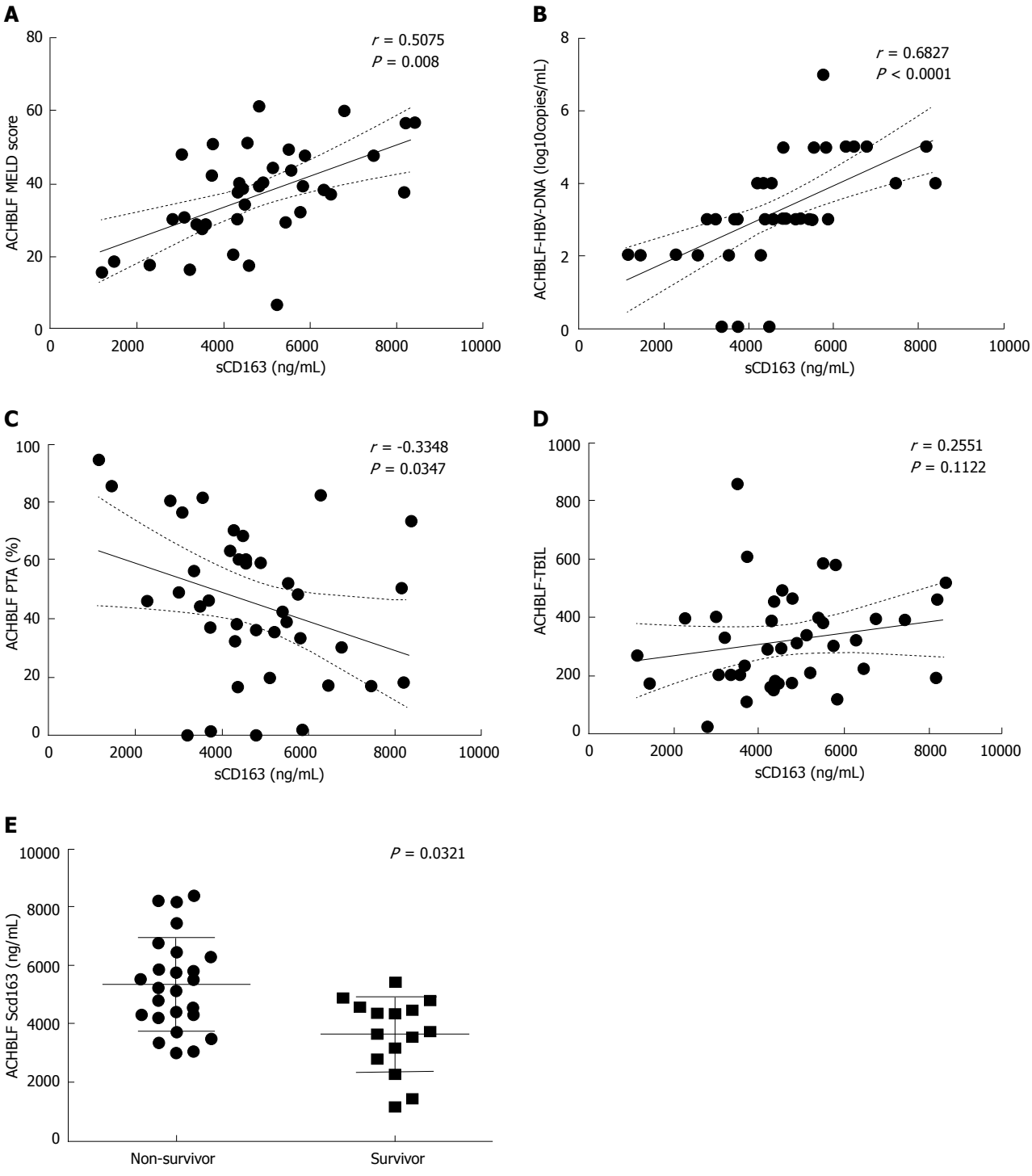

Figure 3 Linear correlation of plasma CD163 levels with disease severity markers in acute-on-chronic hepatitis B liver failure patients. A-C: Plasma CD163 was positively correlated with model of end stage liver disease (MELD) score, hepatitis B virus (HBV)-DNA and negatively correlated with prothrombin activity (PTA); D: There was no correlation between plasma CD163 levels and total bilirubin (TBIL); E: In acute-on-chronic hepatitis B liver failure (ACHBLF) patients, non-survivors had elevated plasma CD163 levels compared with survivors.

ing disease progression during host immune responses caused by the infection in ACHBLF patients. The exact role of HBV in CD163 immune response requires further clarification[34-36].

The present study has limitations, and it would be very interesting to determine the dynamic and histologic expression of CD163 in a future study.

In conclusion, our study demonstrated three major findings. First, the population of CD163+ PBMCs was significantly greater in ACHBLF patients than in CHB patients and healthy controls. Second, CD163 mRNA expression in ACHBLF patients was significantly increased compared with CHB patients and healthy controls. Third, plasma sCD163 levels were markedly increased and cor-

related with disease severity and prognosis in ACHBLF patients. Our findings indicate that CD163 and sCD163 may serve as useful biomarkers and new therapeutic targets for ACHBLF.

COMMENTS

Background

Acute-on-chronic hepatitis B liver failure (ACHBLF) constitutes about 70% of all acute-on-chronic liver failure (ACLF) in areas with a high incidence of hepatitis B. ACHBLF has an extremely poor prognosis due to a lack of understanding of its pathogenesis.

Research frontiers

ACHBLF has an extremely poor prognosis due to a lack of understanding of its pathogenesis. It is important to fully understand the course of the immune pathogenesis of ACHBLF. It may be very helpful to identify a new biomarker which indicates the prognosis of ACHBLF.

Innovations and breakthroughs

This is the first study on the frequency of CD163+ peripheral blood mononuclear cells and CD163 mRNA level in ACHBLF patients. Authors found that increased plasma soluble CD163 (sCD163) levels were correlated with disease severity in ACHBLF patients. These findings strongly suggest that the role of CD163 and sCD163 in the immune response might affect the prognosis of ACHBLF.

Applications

Their findings indicate that CD163 and sCD163 may serve as useful biomarkers and new therapeutic targets for ACHBLF. These biomarkers should be determined as early as possible in ACHBLF patients.

Terminology

ACLF was first used in 1995 to describe a rapid deterioration of liver function due to acute insult to the liver in patients with ongoing or chronic liver disease. Diagnosis of ACLF may be based on the following: acute hepatic insult manifesting as jaundice (serum bilirubin $\geqslant$ 85 μmol/L and coagulopathy international normalized ratio > 1.5 or prothrombin activity < 40%) complicated within 4 wk by ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease. ACHBLF is ACLF with acute or chronic hepatitis B.

Peer review

Overall, this paper is of a very good quality. Study design is appropriate and clear, and helps to answer a clinical relevant question. Data analysis is sufficient. Manuscript is well structured, language is of sufficient quality.

REFERENCES

1 **Kitab B**, Essaid El Feydi A, Afifi R, Trepo C, Benazzouz M, Essamri W, Zoulim F, Chemin I, Alj HS, Ezzikouri S, Benjelloun S. Variability in the precore and core promoter regions of HBV strains in Morocco: characterization and impact on liver disease progression. *PLoS One* 2012; **7**: e42891 [PMID: 22905181 DOI: 10.1371/journal.pone.0042891]

2 **Sarin SK**, Kumar A, Almeida JA, Chawla YK, Fan ST, Garg H, de Silva HJ, Hamid SS, Jalan R, Komolmit P, Lau GK, Liu Q, Madan K, Mohamed R, Ning Q, Rahman S, Rastogi A, Riordan SM, Sakhuja P, Samuel D, Shah S, Sharma BC, Sharma P, Takikawa Y, Thapa BR, Wai CT, Yuen MF. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the study of the liver (APASL). *Hepatol Int* 2009; **3**: 269-282 [PMID: 19669378 DOI: 10.1007/s12072-008-9106-x]

3 **Huang K**, Hu JH, Wang HF, He WP, Chen J, Duan XZ, Zhang AM, Liu XY. Survival and prognostic factors in hepatitis B virus-related acute-on-chronic liver failure. *World J Gastroenterol* 2011; **17**: 3448-3452 [PMID: 21876637 DOI: 10.3748/wjg.v17.i29.3448]

4 **Wang LY**, Meng QH, Zou ZQ, Fan YC, Han J, Qi ZX, Ge J, Xu AL, Wang SK, Wang K. Increased frequency of circulating Th17 cells in acute-on-chronic hepatitis B liver failure. *Dig Dis Sci* 2012; **57**: 667-674 [PMID: 21984439 DOI: 10.1007/s10620-011-1930-5]

5 **Cassetta L**, Cassol E, Poli G. Macrophage polarization in health and disease. *ScientificWorldJournal* 2011; **11**: 2391-2402 [PMID: 22194670 DOI: 10.1100/2011/213962]

6 **Kowal K**, Silver R, Sławińska E, Bielecki M, Chyczewski L, Kowal-Bielecka O. CD163 and its role in inflammation. *Folia Histochem Cytobiol* 2011; **49**: 365-374 [PMID: 22038213]

7 **Lin LN**, Zhu Y, Che FB, Gu JL, Chen JH. Invasive fungal infections secondary to acute-on-chronic liver failure: a retrospective study. *Mycoses* 2013 Feb 1; Epub ahead of print [PMID: 23368965 DOI: 10.1111/myc.12044]

8 **Zhang Z**, Zou ZS, Fu JL, Cai L, Jin L, Liu YJ, Wang FS. Severe dendritic cell perturbation is actively involved in the pathogenesis of acute-on-chronic hepatitis B liver failure. *J Hepatol* 2008; **49**: 396-406 [PMID: 18644645 DOI: 10.1016/j.jhep.2008.05.017]

9 **Yang B**, Wang Y, Zhao C, Yan W, Che H, Shen C, Zhao M. Increased Th17 cells and interleukin-17 contribute to immune activation and disease aggravation in patients with chronic hepatitis B virus infection. *Immunol Lett* 2013; **149**: 41-49 [PMID: 23237940 DOI: 10.1016/j.imlet.2012.12.001]

10 **Burdo TH**, Lo J, Abbara S, Wei J, DeLelys ME, Preffer F, Rosenberg ES, Williams KC, Grinspoon S. Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients. *J Infect Dis* 2011; **204**: 1227-1236 [PMID: 21917896 DOI: 10.1093/infdis/jir520]

11 **Fabriek BO**, van Bruggen R, Deng DM, Ligtenberg AJ, Nazmi K, Schornagel K, Vloet RP, Dijkstra CD, van den Berg TK. The macrophage scavenger receptor CD163 functions as an innate immune sensor for bacteria. *Blood* 2009; **113**: 887-892 [PMID: 18849484 DOI: 10.1182/blood-2008-07-167064]

12 **Shephard DA**. The 1975 Declaration of Helsinki and consent. *Can Med Assoc J* 1976; **115**: 1191-1192 [PMID: 1000449]

13 **Etzerodt A**, Maniecki MB, Graversen JH, Møller HJ, Torchilin VP, Moestrup SK. Efficient intracellular drug-targeting of macrophages using stealth liposomes directed to the hemoglobin scavenger receptor CD163. *J Control Release* 2012; **160**: 72-80 [PMID: 22306335 DOI: 10.1016/j.jconrel.2012.01.034]

14 **Tippett E**, Cheng WJ, Westhorpe C, Cameron PU, Brew BJ, Lewin SR, Jaworowski A, Crowe SM. Differential expression of CD163 on monocyte subsets in healthy and HIV-1 infected individuals. *PLoS One* 2011; **6**: e19968 [PMID: 21625498 DOI: 10.1371/journal.pone.0019968]

15 **Higashi-Kuwata N**, Jinnin M, Makino T, Fukushima S, Inoue Y, Muchemwa FC, Yonemura Y, Komohara Y, Takeya M, Mitsuya H, Ihn H. Characterization of monocyte/macrophage subsets in the skin and peripheral blood derived from patients with systemic sclerosis. *Arthritis Res Ther* 2010; **12**: R128 [PMID: 20602758 DOI: 10.1186/ar3066]

16 **He WP**, Hu JH, Zhao J, Tong JJ, Ding JB, Lin F, Wang HF. Comparison of four prognostic models and a new Logistic regression model to predict short-term prognosis of acute-on-chronic hepatitis B liver failure. *Chin Med J* (Engl) 2012; **125**: 2272-2278 [PMID: 22882847]

17 **Zheng MH**, Shi KQ, Fan YC, Li H, Ye C, Chen QQ, Chen YP. A model to determine 3-month mortality risk in patients with acute-on-chronic hepatitis B liver failure. *Clin Gastroenterol Hepatol* 2011; **9**: 351-356.e3 [PMID: 21195790 DOI: 10.1016/j.cgh.2010.12.027]

18 **Møller HJ**. Soluble CD163. *Scand J Clin Lab Invest* 2012; **72**: 1-13 [PMID: 22060747 DOI: 10.3109/00365513.2011.626868]

19 **Møller HJ**, Grønbaek H, Schiødt FV, Holland-Fischer P, Schilsky M, Munoz S, Hassanein T, Lee WM. Soluble CD163 from activated macrophages predicts mortality in acute liver failure. *J Hepatol* 2007; **47**: 671-676 [PMID: 17629586]

20 **Shabo I**, Svanvik J. Expression of macrophage antigens by tumor cells. *Adv Exp Med Biol* 2011; **714**: 141-150 [PMID: 21506012 DOI: 10.1007/978-94-007-0782-5_7]

21 **Onofre G**, Kolácková M, Jankovicová K, Krejsek J. Scavenger receptor CD163 and its biological functions. *Acta Medica* (Hradec Kralove) 2009; **52**: 57-61 [PMID: 19777868]

22 **Davis BH**, Zarev PV. Human monocyte CD163 expression inversely correlates with soluble CD163 plasma levels. *Cytometry B Clin Cytom* 2005; **63**: 16-22 [PMID: 15624200]

23 **Hintz KA**, Rassias AJ, Wardwell K, Moss ML, Morganelli PM, Pioli PA, Givan AL, Wallace PK, Yeager MP, Guyre PM. Endotoxin induces rapid metalloproteinase-mediated shedding followed by up-regulation of the monocyte hemoglobin scavenger receptor CD163. *J Leukoc Biol* 2002; **72**: 711-717 [PMID: 12377940]

24 **Weaver LK**, Pioli PA, Wardwell K, Vogel SN, Guyre PM. Up-regulation of human monocyte CD163 upon activation of cell-surface Toll-like receptors. *J Leukoc Biol* 2007; **81**: 663-671 [PMID: 17164428]

25 **Kneidl J**, Löffler B, Erat MC, Kalinka J, Peters G, Roth J, Barczyk K. Soluble CD163 promotes recognition, phagocytosis and killing of Staphylococcus aureus via binding of specific fibronectin peptides. *Cell Microbiol* 2012; **14**: 914-936 [PMID: 22309204 DOI: 10.1111/j.1462-5822.2012.01766.x]

26 **Piatkowski A**, Grieb G, Das R, Bozkurt A, Ulrich D, Pallua N. Soluble CD163: A novel biomarker for the susceptibility to sepsis in severe burn injuries. *Indian J Plast Surg* 2011; **44**: 118-124 [PMID: 21713197 DOI: 10.4103/0970-0358.81454]

27 **Burdo TH**, Lentz MR, Autissier P, Krishnan A, Halpern E, Letendre S, Rosenberg ES, Ellis RJ, Williams KC. Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after anti-retroviral therapy. *J Infect Dis* 2011; **204**: 154-163 [PMID: 21628670 DOI: 10.1093/infdis/jir214]

28 **Jude C**, Dejica D, Samasca G, Balacescu L, Balacescu O. Soluble CD163 serum levels are elevated and correlated with IL-12 and CXCL10 in patients with long-standing rheumatoid arthritis. *Rheumatol Int* 2013; **33**: 1031-1037 [PMID: 23011084]

29 **Fan HL**, Yang PS, Chen HW, Chen TW, Chan DC, Chu CH, Yu JC, Kuo SM, Hsieh CB. Predictors of the outcomes of acute-on-chronic hepatitis B liver failure. *World J Gastroenterol* 2012; **18**: 5078-5083 [PMID: 23049217 DOI: 10.3748/wjg.v18.i36.5078]

30 **Feng L**, Zhou X, Su LX, Feng D, Jia YH, Xie LX. Clinical significance of soluble hemoglobin scavenger receptor CD163 (sCD163) in sepsis, a prospective study. *PLoS One* 2012; **7**: e38400 [PMID: 22911680 DOI: 10.1371/journal.pone.0038400]

31 **Matsushita N**, Kashiwagi M, Wait R, Nagayoshi R, Nakamura M, Matsuda T, Hogger P, Guyre PM, Nagase H, Matsuyama T. Elevated levels of soluble CD163 in sera and fluids from rheumatoid arthritis patients and inhibition of the shedding of CD163 by TIMP-3. *Clin Exp Immunol* 2002; **130**: 156-161 [PMID: 12296867]

32 **Yan Y**, Mai L, Zheng YB, Zhang SQ, Xu WX, Gao ZL, Ke WM. What MELD score mandates use of entecavir for ACLF-HBV HBeAg-negative patients? *World J Gastroenterol* 2012; **18**: 4604-4609 [PMID: 22969236 DOI: 10.3748/wjg.v18.i33.4604]

33 **Zhang Z**, Zhang JY, Wang LF, Wang FS. [The relationship of HBeAg status with HBV DNA loads, MELD scores in patients with acute-on-chronic hepatitis B liver failure during terminal phases]. *Zhonghua Shiyan He Linchuang Bingduxue Zazhi* 2012; **26**: 51-53 [PMID: 22919755]

34 **Valisena S**, Palumbo M, Parolin C, Palú G, Meloni GA. Relevance of ionic effects on norfloxacin uptake by Escherichia coli. *Biochem Pharmacol* 1990; **40**: 431-436 [PMID: 2200406 DOI: 10.1111/j.1440-1746.2011.06940.x]

35 **Su L**, Feng L, Liu C, Jiang Z, Li M, Xiao K, Yan P, Jia Y, Feng D, Xie L. Diagnostic value of urine sCD163 levels for sepsis and relevant acute kidney injury: a prospective study. *BMC Nephrol* 2012; **13**: 123 [PMID: 23013330 DOI: 10.1186/1471-2369-13-123]

36 **Ingels C**, Møller HJ, Hansen TK, Wouters PJ, Vanhorebeek I, Van den Berghe G. Circulating levels of the shed scavenger receptor sCD163 and association with outcome of critically ill patients. *J Clin Immunol* 2013; **33**: 619-629 [PMID: 23150181 DOI: 10.1007/s10875-012-9830-9]

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CASE REPORT

Massive hepatic necrosis with toxic liver syndrome following portal vein ligation

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Abstract

Right portal vein ligation (PVL) is a safe and widespread procedure to induce controlateral liver hypertrophy for the treatment of bilobar colorectal liver metastases. We report a case of a 60-year-old man treated by both right PVL and ligation of the glissonian branches of segment 4 for colorectal liver metastases surrounding the right and median hepatic veins. After surgery, the patient developed massive hepatic necrosis with secondary pulmonary and renal insufficiency requiring transfer to the intensive care unit. This so-called toxic liver syndrome finally regressed after hemofiltration and positive oxygen therapy. Diagnosis of acute congestion of the ligated lobe was suspected. The mechanism suspected was an increase in arterial inflow secondary to portal vein ligation concomitant with a decrease in venous outflow due to liver metastases encircling the right and median hepatic vein. This is the first documented case of toxic liver syndrome in a non-cirrhotic patient with favorable issue, and a rare complication of PVL.

Key words: Colorectal liver metastases; Portal vein ligation; Liver failure; Toxic liver syndrome; Hemofiltration

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INTRODUCTION

Portal vein embolization (PVL) was first used by Makuuchi *et al*[1] to induce liver hypertrophy before major hepatectomy in biliary cancers. The technique was then applied to hepatocellular carcinoma and colorectal liver metastases (CRLM) to decrease postoperative morbidity and mortality[2,3]. PVL and portal vein embolization (PVE) are generally safe with few side effects in non-cirrhotic patients[4]. Suppression of the right portal flow causes atrophy of the right lobe, and induces the production of various growth factors and proinflammatory cytokines that prepare hepatocytes for growth (for review, see[5]). Whereas arterial ischemia can induce massive liver necrosis, the suppression of portal flow leads to progressive apoptosis with minor consequences for liver function[6], owing mainly to a compensatory increase in arterial blood flow in the deprived lobe, a phenomenon called "hepatic arterial buffer response (HABR)"[7]. Here we report a case of massive hepatic necrosis of both the right lobe

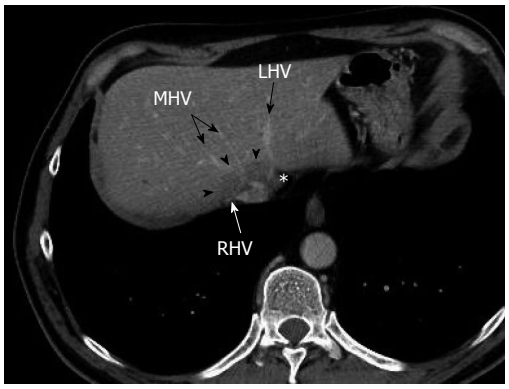

Figure 1 Preoperative multidetector computed tomograph at portal venous phase showing a large metastasis (arrowheads) encircling the confluence of the right hepatic vein and middle hepatic vein, without any thrombosis, and displacing the end of the left hepatic vein (asterisk). RHV: Right hepatic vein; MHV: Middle hepatic vein; LHV: Left hepatic vein.

and segment 4 after PVL in a patient treated for CRLM, with secondary cardiac, pulmonary and renal insufficiency due to toxic liver syndrome.

CASE REPORT

A 60-year-old man was referred to our tertiary center for treatment of metachronous bilobar colorectal liver metastases. He had no medical history. Two years before he had undergone right colectomy for stage II colon cancer with no adjuvant anticancer therapy. During oncologic follow-up, three CRLM were detected by multidetector computed tomography scan: two in the right lobe, and one large one encircling the right and middle hepatic veins and displacing the left hepatic vein (Figure 1). Neoadjuvant chemotherapy (six courses of FOLFOX with cetuximab) was administered, with control imaging showing objective response according to RECIST criteria[8]. Radical resection required right hepatectomy enlarged to segment 4 (right trisectionectomy according to the Brisbane terminology[9]). To improve left lobe hypertrophy, we decided to perform a two-stage surgical procedure: first, right PVL with ligation of the glissonian branches of segment 4, and assessment of resectability using intraoperative liver ultrasonography (US) followed by right trisectionectomy. At first stage laparotomy, the liver was normal with no sign of chemotherapy-related injury. CRLM were confined to the right lobe and did not involve the left hepatic vein. A right PVL was performed with ligation of the glissonian branches of segment 4.

The immediate postoperative course was marked by a massive peak of transaminase and moderate liver insufficiency (Figure 2). The patient developed pulmonary and renal insufficiency with oligoanuria necessitating transfer to the intensive care unit (ICU) for non-invasive positive pressure ventilation and hemofiltration. US of the hepatic pedicle did not show any vascular thrombosis. Multidetector computed tomography (MD-CT) performed at postoperative day (POD) 2 showed massive hepatic necrosis of segment 4 and of a large part of the right hemi-

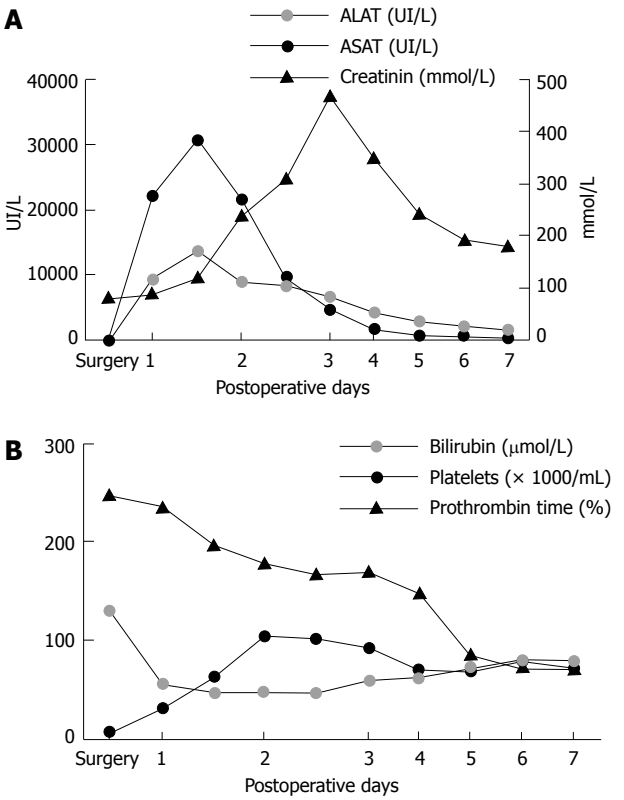

Figure 2 Postoperative blood tests. A: Transaminases increased from postoperative day 1, reached a peak at postoperative day 3, then returned to normal value on postoperative day 7. Serum creatinin started to increase after the peak of transaminases, as observed in the toxic liver syndrome, and decreased after hemofiltration; B: Platelets decreased regularly as from the first postoperative day. Total bilirubin increased from the first postoperative day and reached a plateau around 100 μmol/L. Prothrombin time fell rapidly to 47% at postoperative day 1 and thereafter increased progressively. ASAT: Aspartate amino transferase; ALAT: Alanine amino transferase.

liver, hypertrophy of the left lobe and ascites (Figure 3). Renal biopsy revealed acute tubular necrosis without any particularity (Figure 4). Liver biopsy was not performed because of relative liver insufficiency and ascites. The level of transaminases started to decrease progressively from POD 2, and hemofiltration was stopped after recovery of renal function at POD 12. Finally, the patient was discharged from ICU at POD 15. A liver abscess developed in segment 8 near the necrotic area. Percutaneous drainage showed infected collection without biliary leak, with favorable outcome after appropriate antibiotic treatment. The patient was discharged from hospital after 52 d.

DISCUSSION

Portal vein occlusion by ligation or embolization is a safe procedure widely used to induce liver hypertrophy of the future remnant liver before major liver resection. The mortality of this procedure is these procedures nil and major morbidity is about 1%[6]. In the event of post-procedure symptoms, which occur in more than 50% of patients, transaminase levels peak at a level less than three times baseline 1 to 3 d after embolization and return to baseline in 7-10 d[10]. Fever and post-embolization syn-

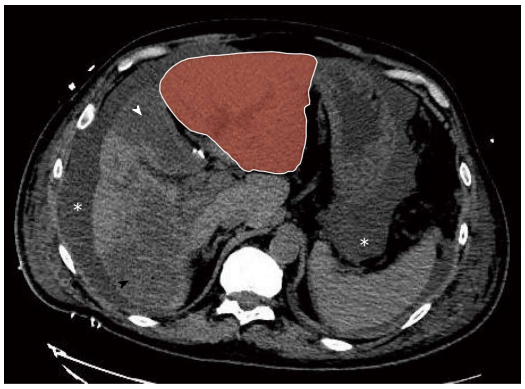

Figure 3 Postoperative day 2 non-enhanced multidetector computed tomograph scan. Massive hepatic necrosis of the medial segment of the left lobe (segment 4, white arrowhead) and of a large part of the right lobe (black arrowhead) are shown, with remarkable hypertrophy of the lateral segments of the left lobe (red zone). Note also ascites (asterisk).

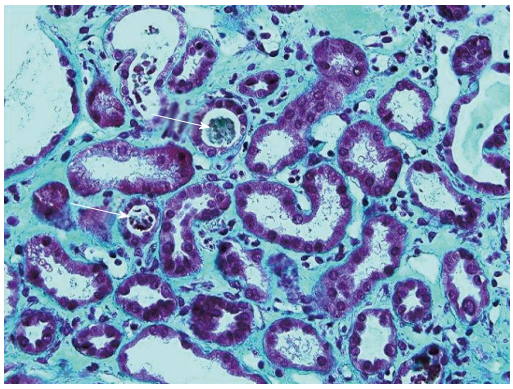

Figure 4 Renal biopsy showing acute tubular necrosis: Tubules dilated with sometimes a low-lying epithelium and pleomorphic nuclei. The brush border of the proximal tubular cells is missing. The lumen of some tubules contains rare tubular cell necrosis (white arrows). There is a diffuse interstitial edema (Masson's trichrome, ×40).

drome, which are frequently observed after trans-arterial chemoembolization, are rare in PVE or PVL. Markers of liver insufficiency (bilirubin, prothombin time) are usually not affected. Hepatic necrosis of the ligated lobe has never been reported as a major complication[4]. Despite having different courses within the liver, arterial and portal blood flow drain in a common terminal hepatic veinule[11]. Thus, occlusion of a branch of the portal vein induces an ipsilateral HABR that guarantees rapid normalization of overall blood flow in the ligated lobe[12]. Flow in the hepatic artery of the ligated lobe increases by more than 3-fold for this compensatory effect_and is usually well tolerated as venous outflow can absorb the overflow[13]. Hepatic necrosis involves the interruption of hepatic arterial flow with secondary damage due to liver hypoxemia. Interestingly, portal arterialization by arterioportal shunting restores normal oxygen supply within the liver and can prevent liver necrosis[14]. Hence, hypoxemia is the main cause of liver necrosis and explains why portal vein obstruction alone cannot induce hepatic necrosis. In contrast, impairment of venous outflow following compression by liver tumors may be very deleterious in this situation. Studies on venous drainage of right lobe grafts in living donor liver transplantation have demonstrated that non reconstruction of the middle hepatic vein can lead to congestion of the anterior sector, with possible severe liver dysfunction[15,16]. In such cases, assessment of hepatic tissue oxygenation using near-infrared spectrometry has confirmed congestion with hypoxemia of the anterior sector[17]. As a consequence, portal veins can become the draining veins to ensure adequate venous outflow, *via* a mechanism of regurgitation[18]. Finally, it can be speculated that compression of the right and median hepatic veins by liver metastases silently reduced the flow in these veins, with partial recovery of liver outflow through the portal system. PVL in this condition could have induced acute congestion with secondary necrosis of the right liver. Congestion was probably worsened by increased hepatic arterial inflow due to hepatic arterial buffer response secondary to PVL.

Acute congestion probably led to massive hepatic necrosis of the right lobe with partial liver insufficiency. In our patient, massive hepatic necrosis caused renal, cardiac and respiratory dysfunction. This so-called "toxic liver syndrome (TLS)" was first described by Ringe *et al*[19] in fulminant hepatic failure. Definition is based on complete liver necrosis associated with cardiovascular shock, renal, and possibly respiratory failure requiring vasopressor support, hemodialysis, and mechanical ventilation[19]. The pathophysiology is still unclear but seems to involve toxic metabolites released from the necrotic liver, such as cytokines or cardiosuppressive factors, known to play a role in cardiac and pulmonary instability after liver ischemia-reperfusion syndrome[20,21]. Furthermore, intra-abdominal hypertension related to an increase in the volume of the intra-abdominal organs and ascites can impair cardiac preload by reduced venous return and pulmonary function and by limitation of abdominal wall expansion[22]. TLS is invariably fatal, and all the studies on the topic have been published in the setting of two-stage liver transplantation[19,23-26]. To the best of our knowledge, there are no documented cases of reversible TLS. The syndrome usually develops following graft failure or acute rejection in liver transplantation, but can also have traumatic, toxic or postoperative causes[19]. Spontaneous evolution of TLS is fatal mainly because of its association with liver failure[19]. One approach is wo-stage liver transplantation, which consists in (1) removing the necrotic liver to avoid toxic syndrome and stabilize the patient; and (2) restoring hepatic function with transplantation[27]. In our case, the patient did not develop fatal complications, for at least two reasons. First, only a part of the liver was involved by necrosis so that TLS was comparatively moderate. Second, the remnant liver spared by necrosis was neither cirrhotic nor fibrotic and was able to ensure sufficient hepatic function to minimize liver insufficiency, as demonstrated by the rapid hypertrophy visualized on MDCT. Taken together, these two factors may have limited the consequences of TLS and directly contributed to the patient's favorable clinical evolution.

Portal vein ligation must be avoided when impaired venous return is suspected. There is the risk of massive

hepatic necrosis occurring due to hepatic congestion and secondary toxic liver syndrome, which can be fatal. This is the first documented observation of a spontaneously reversible toxic liver syndrome with minor hepatic failure.

REFERENCES

1 **Makuuchi M**, Thai BL, Takayasu K, Takayama T, Kosuge T, Gunvén P, Yamazaki S, Hasegawa H, Ozaki H. Preoperative portal embolization to increase safety of major hepatectomy for hilar bile duct carcinoma: a preliminary report. *Surgery* 1990; **107**: 521-527 [PMID: 2333592]

2 **Kinoshita H**, Sakai K, Hirohashi K, Igawa S, Yamasaki O, Kubo S. Preoperative portal vein embolization for hepatocellular carcinoma. *World J Surg* 1986; **10**: 803-808 [PMID: 3022488 DOI: 10.1007/BF01655244]

3 **Kawasaki S**, Makuuchi M, Kakazu T, Miyagawa S, Takayama T, Kosuge T, Sugihara K, Moriya Y. Resection for multiple metastatic liver tumors after portal embolization. *Surgery* 1994; **115**: 674-677 [PMID: 8197557]

4 **Abulkhir A**, Limongelli P, Healey AJ, Damrah O, Tait P, Jackson J, Habib N, Jiao LR. Preoperative portal vein embolization for major liver resection: a meta-analysis. *Ann Surg* 2008; **247**: 49-57 [PMID: 18156923 DOI: 10.1097/SLA.0b013e31815f6e5b]

5 **Yokoyama Y**, Nagino M, Nimura Y. Mechanisms of hepatic regeneration following portal vein embolization and partial hepatectomy: a review. *World J Surg* 2007; **31**: 367-374 [PMID: 17219273 DOI: 10.1007/s00268-006-0526-2]

6 **Ebata T**, Yokoyama Y, Igami T, Sugawara G, Takahashi Y, Nagino M. Portal vein embolization before extended hepatectomy for biliary cancer: current technique and review of 494 consecutive embolizations. *Dig Surg* 2012; **29**: 23-29 [PMID: 22441616 DOI: 10.1159/000335718]

7 **Lautt WW**. Mechanism and role of intrinsic regulation of hepatic arterial blood flow: hepatic arterial buffer response. *Am J Physiol* 1985; **249**: G549-G556 [PMID: 3904482]

8 **Therasse P**, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; **92**: 205-216 [PMID: 10655437 DOI: 10.1093/jnci/92.3.205]

9 **Strasberg S**, Belghiti J, Clavien P, Gadzijev E, Garden J, Lau W. The Brisbane 2000 terminology of liver anatomy and resections. *HPB* (Oxford) 2000; **2**: 333-339 [DOI: 10.1080/136518202760378489]

10 **Liu H**, Zhu S. Present status and future perspectives of preoperative portal vein embolization. *Am J Surg* 2009; **197**: 686-690 [PMID: 19249737 DOI: 10.1016/j.amjsurg.2008.04.022]

11 **Sherman IA**, Dlugosz JA, Barker F, Sadeghi FM, Pang KS. Dynamics of arterial and portal venous flow interactions in perfused rat liver: an intravital microscopic study. *Am J Physiol* 1996; **271**: G201-G210 [PMID: 8760124]

12 **Richter S**, Vollmar B, Mücke I, Post S, Menger MD. Hepatic arteriolo-portal venular shunting guarantees maintenance of nutritional microvascular supply in hepatic arterial buffer response of rat livers. *J Physiol* 2001; **531**: 193-201 [PMID: 11179403 DOI: 10.1111/j.1469-7793.2001.0193j.x]

13 **Kollmar O**, Corsten M, Scheuer C, Vollmar B, Schilling MK, Menger MD. Portal branch ligation induces a hepatic arterial buffer response, microvascular remodeling, normoxygenation, and cell proliferation in portal blood-deprived liver tissue. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G1534-G1542 [PMID: 17347450 DOI: 10.1152/ajpgi.00503.2006]

14 **Shimizu Y**, Miyazaki M, Shimizu H, Ito H, Nakagawa K, Ambiru S, Yoshidome H, Nakajima N. Beneficial effects of arterialization of the portal vein on extended hepatectomy. *Br J Surg* 2000; **87**: 784-789 [PMID: 10848860 DOI: 10.1046/j.1365-2168.2000.01382.x]

15 **Lee S**, Park K, Hwang S, Lee Y, Choi D, Kim K, Koh K, Han S, Choi K, Hwang K, Makuuchi M, Sugawara Y, Min P. Congestion of right liver graft in living donor liver transplantation. *Transplantation* 2001; **71**: 812-814 [PMID: 11330547 DOI: 10.1097/00007890-200103270-00021]

16 **Yamamoto H**, Maetani Y, Kiuchi T, Ito T, Kaihara S, Egawa H, Itoh K, Kamiyama Y, Tanaka K. Background and clinical impact of tissue congestion in right-lobe living-donor liver grafts: a magnetic resonance imaging study. *Transplantation* 2003; **76**: 164-169 [PMID: 12865804 DOI: 10.1097/01.TP.0000072340.87482.17]

17 **Cui D**, Kiuchi T, Egawa H, Hayashi M, Sakamoto S, Ueda M, Kaihara S, Uemoto S, Inomata Y, Tanaka K. Microcirculatory changes in right lobe grafts in living-donor liver transplantation: a near-infrared spectrometry study. *Transplantation* 2001; **72**: 291-295 [PMID: 11477355 DOI: 10.1097/00007890-200107270-00022]

18 **Murata S**, Itai Y, Asato M, Kobayashi H, Nakajima K, Eguchi N, Saida Y, Kuramoto K, Tohno E. Effect of temporary occlusion of the hepatic vein on dual blood in the liver: evaluation with spiral CT. *Radiology* 1995; **197**: 351-356 [PMID: 7480676]

19 **Ringe B**, Lübbe N, Kuse E, Frei U, Pichlmayr R. Total hepatectomy and liver transplantation as two-stage procedure. *Ann Surg* 1993; **218**: 3-9 [PMID: 8328827 DOI: 10.1097/00000658-199307000-00002]

20 **Bihari DJ**, Gimson AE, Williams R. Cardiovascular, pulmonary and renal complications of fulminant hepatic failure. *Semin Liver Dis* 1986; **6**: 119-128 [PMID: 3529406 DOI: 10.1055/s-2008-1040595]

21 **Blanot S**, Gillon MC, Lopez I, Ecoffey C. Circulating endotoxins and postreperfusion syndrome during orthotopic liver transplantation. *Transplantation* 1995; **60**: 103-106 [PMID: 7624932 DOI: 10.1097/00007890-199507150-00019]

22 **Joshi D**, Saha S, Bernal W, Heaton N, Wendon J, Auzinger G. Haemodynamic response to abdominal decompression in acute Budd-Chiari syndrome. *Liver Int* 2011; **31**: 1171-1178 [PMID: 21745299 DOI: 10.1111/j.1478-3231.2011.02557.x]

23 **Domínguez Fernández E**, Lange K, Lange R, Eigler FW. Relevance of two-stage total hepatectomy and liver transplantation in acute liver failure and severe liver trauma. *Transpl Int* 2001; **14**: 184-190 [PMID: 11499909 DOI: 10.1111/j.1432-2277.2001.tb00039.x]

24 **Guirl MJ**, Weinstein JS, Goldstein RM, Levy MF, Klintmalm GB. Two-stage total hepatectomy and liver transplantation for acute deterioration of chronic liver disease: a new bridge to transplantation. *Liver Transpl* 2004; **10**: 564-570 [PMID: 15048803 DOI: 10.1002/lt.20134]

25 **Ringe B**, Pichlmayr R. Total hepatectomy and liver transplantation: a life-saving procedure in patients with severe hepatic trauma. *Br J Surg* 1995; **82**: 837-839 [PMID: 7627526 DOI: 10.1002/bjs.1800820637]

26 **Ferraz-Neto BH**, Moraes-Junior JM, Hidalgo R, Zurstrassen MP, Lima IK, Novais HS, Rezende MB, Meira-Filho SP, Afonso RC. Total hepatectomy and liver transplantation as a two-stage procedure for toxic liver: case reports. *Transplant Proc* 2008; **40**: 814-816 [PMID: 18455026 DOI: 10.1016/j.transproceed.2008.02.043]

27 **Ringe B**, Pichlmayr R, Lübbe N, Bornscheuer A, Kuse E. Total hepatectomy as temporary approach to acute hepatic or primary graft failure. *Transplant Proc* 1988; **20**: 552-557 [PMID: 3279648]

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CASE REPORT

Polyarteritis nodosa diagnosed by surgically resected jejunal necrosis following acute abdomen

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Abstract

The differential diagnosis of acute abdomen is typically extremely broad in range, with vasculitis posing a rare but potentially life-threatening cause of acute abdomen. Here, we report a case of acute abdomen with bowel wall thickening limited to jejunum, accompanied by unexplained renal dysfunction. Later, the patient was diagnosed as having polyarteritis nodosa based on surgically resected jejunal necrosis. Despite aggressive treatment, including the use of steroid pulse therapy and continuous hemodiafiltration, the patient died. Although polyarteritis nodosa is extremely rare in patients with acute abdomen, acute abdomen is relatively common manifestation of that. And it is reported that involvement of small intestine suggests poorer prognosis. Our case highlights the importance of vasculitis as a differential diagnosis of patients with atypical acute abdomen. In this report, we not only review possible clues that might have led to an earlier diagnosis in this case, but also attempt to draw some lessons for treating similar cases in the future.

Key words: Acute abdomen; Polyarteritis nodosa; Jejunal necrosis

Core tip: Our case shows the importance of vasculitis, including polyarteritis nodosa, as a differential diagnosis in case of acute abdomen. Here we provide comprehensive review of gastrointestinal organ involvement in Polyarteritis nodosa, and concluded that gastrointestinal lesions, especially small intestinal lesion, is relatively common manifestation and that suggests high mortality. Then we draw two findings, bowel wall thickening limited to jejunum and unexplained renal dysfunction, as possible clues that might led us to earlier diagnosis in this case. Additionally, we discuss possible relationship between pathophysiology of intestinal ischemia and radiological findings.

Hiraike Y, Kodaira M, Sano M, Terazawa Y, Yamagata S, Terada S, Ohura M, Kuriki K. Polyarteritis nodosa diagnosed by surgically resected jejunal necrosis following acute abdomen. *World J Gastroenterol* 2013; 19(18): 2830-2834 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i18/2830.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i18.2830

INTRODUCTION

Polyarteritis nodosa is a systemic vasculitis affecting medium- and small-sized arteries[1,2]. The affected sites can include the kidneys, gastrointestinal tract, heart, peripheral and central nervous system, and skin. Accurately diagnosing the disease is difficult because there are no

known markers, making biopsy a mandatory procedure. Here we report the case of a patient with acute abdomen diagnosed as polyarteritis nodosa based on surgically resected jejunal necrosis.

CASE REPORT

A 70-year-old female with a history of diabetes mellitus, hypertension, and gallbladder stone visited the emergency department at our institution complaining of severe abdominal pain. Upon examination, she appeared slightly distressed; her temperature was 36.6 ℃, blood pressure 153/101 mmHg, pulse 100 beats/min, and oxygen saturation 98% on room air. Epigastric tenderness without rebound or guarding tenderness, and pitting edema of all extremities were noted. The laboratory findings were remarkable for elevated white blood cell count (20650/μL), C-reactive protein (8.62 mg/dL), and impaired renal function (BUN 70.2 mg/dL, creatinine 2.63 mg/dL). Urinalysis showed 1+ protein and 2+ occult blood. Although her platelet count had fallen to 8.6 × 10^4/μL, coagulation tests were normal. Serologies for hepatitis B virus, hepatitis C virus, anti-nuclear antibodies, cytoplasmic-Anti-Neutrophil Cytoplasmic Antibodies (ANCA), and perinuclear-ANCA were also negative. Abdominal computed tomography (CT) without contrast showed bowel wall thickening almost limited to jejunum, a stone at the neck of gallbladder, and a moderate amount of ascites surrounding the liver and the Douglas's pouch (Figure 1). Based on these findings, the patient was admitted to our institution with a diagnosis of acute abdomen, and intravenous sulbactam/cefoperazone was started.

Although her abdominal pain improved spontaneously after admission, her response to the antibiotic was minimal and her inflammatory reaction remained high. On day 6 following admission, the patient complained of severe abdominal pain. An emergent CT showed deterioration of the edema in the jejunum and the ascites (Figure 1). Although her antibiotic regimen was switched to imipenem/cilastatin, no improvement was observed. In light of these findings, an emergent laparotomy was carried out with tentative diagnoses including a serious intestinal infection refractory to antibiotics, a thrombosis or occlusion of the mesenteric vasculatures, and an intestinal lymphoma. Laparotomy findings included hemorrhagic ascites measuring more than 2000 mL, and a swollen, dark red jejunum. Twenty centimeters of the jejunum was resected, and an end-to-end anastomosis was conducted. The pathology was consistent with a diagnosis of polyarteritis nodosa (Figure 2). The pathological finding of a post-surgical skin lesion on her forearm was also consistent with the diagnosis.

On day 17 following admission, steroid pulse therapy (methylprednisolone 1000 mg/d for 3 d) was prescribed, followed by a maintenance dose of steroids (60 mg/d for 8 d). Although her inflammatory reaction and renal function initially improved, the patient suffered a relapse of disease activity and her general status deteriorated despite continuous hemodiafiltration and extensive use of antibiotics including ceftriaxone and ceftazidime. On day 32 following admission, an acute onset of left hemiparesis was observed, with normal head CT findings (Figure 3). At midnight of the same day, sudden respiratory arrest occurred. Although cardiopulmonary resuscitation was performed immediately after arrest, the patient died. An autopsy was not performed. The clinical course is summarized in Figure 4.

DISCUSSION

Polyarteritis nodosa is a type of vasculitis affecting medium- and small-sized arteries. Epidemiologic studies of polyarteritis nodosa have generally been difficult to interpret because they have typically included patients with microscopic polyarteritis and other possible diseases as well. Recent studies, however, have estimated the prevalence per one million adults to be approximately 30 in France and Sweden[3,4]. Possible symptoms of polyarteritis nodosa include weight loss, livedo reticularis, testicular pain, myalgias or muscle weakness, mono- or polyneuropathy, hypertension, and renal dysfunction[1]. Unfortunately, no definitive diagnostic marker has ever been identified, and diagnosis is most often confirmed by biopsy. The prognosis of polyarteritis nodosa depends on the existence of serious organ involvement; in this regard, Guillevin *et al*[5] have established 5 risk factors leading to higher rates of mortality. These include proteinuria > 1 g/d [relative risk (RR) for death was 3.6], renal insufficiency with serum creatinine > 1.58 mg/dL (RR = 1.86), gastrointestinal tract surgery (RR = 2.83), cardiomyopathy (RR = 2.18), and central nervous system involvement (RR = 1.76). When none of the 5 prognostic factors is present, the mortality rate at 5 years is 11.9%. However, when more than 3 factors are present, the mortality rate is as high as 45.95%.

Although polyarteritis nodosa is extremely rare in patients with an acute abdomen, acute abdomen is a relatively common manifestation of polyarteritis nodosa. Levine *et al*[6] reported that 24 of the 54 cases (44%) they reviewed involved gastrointestinal lesions, and 13 required surgical intervention. Of the 24 cases presenting a gastrointestinal lesion, the mortality rate proved higher in the group requiring surgery (3/13, 23%) compared to the group that received more conservative treatment (1/11, 9%). The small intestine was reported to be involved in 3 of the 9 cases with polyarteritis nodosa that required abdominal surgery, 2 of the 3 patients died in such cases, while just 1 of the 6 patients died when the small intestine was spared[7]. According to an investigation of autopsy summaries in Japan, the small intestine is the most common site of gastrointestinal lesions and it is involved in 12 of the 15 cases (80%), while the large intestine is involved in 10 cases, liver and pancreas in 9 cases, gallbladder in 8 cases, stomach in 7 cases, tongue and esophagus in 6 cases, mesenterium in 3 cases, and appendix in 2 cases[8]. In summary, the small intestine is often involved in polyarteritis nodosa with acute

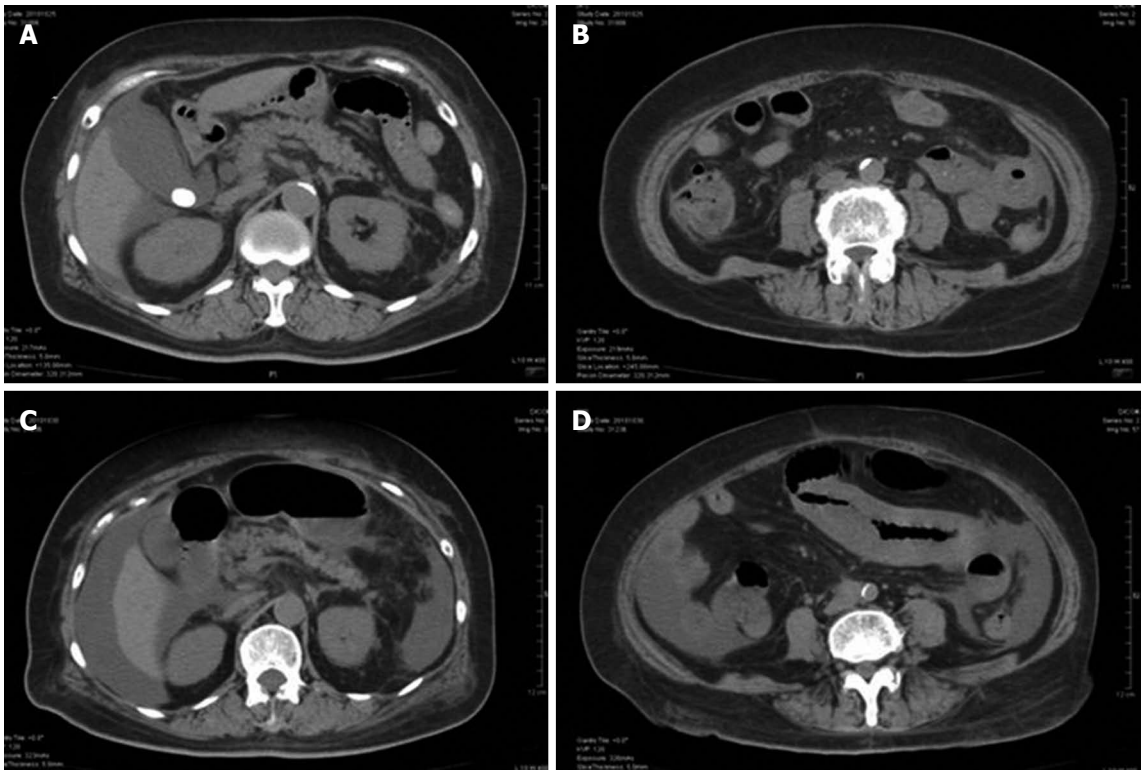

Figure 1 Abdominal computed tomography findings. A, B: On day 1 showed ascites and bowel wall thickening; C, D: On day 6, both of the ascites and the bowel wall thickening, especially jejunum, had severely worsened.

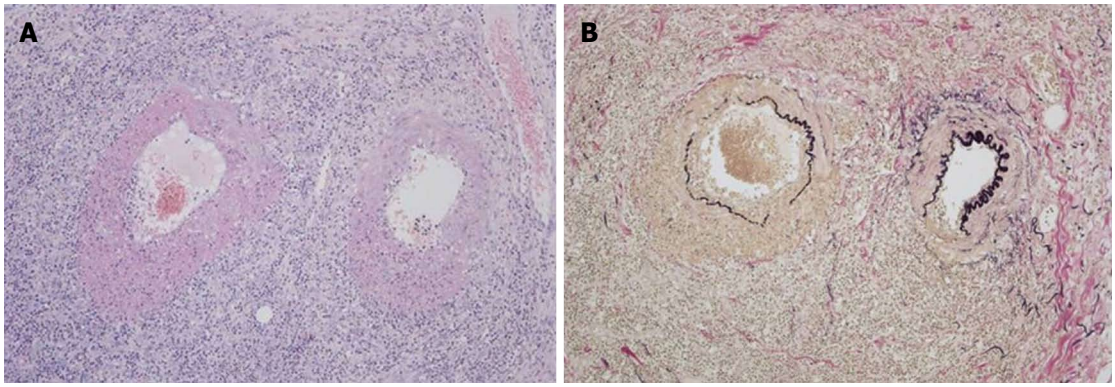

Figure 2 Pathological specimen from the resected jejunum showing fibrinoid necrosis of the arterial wall and infiltration of inflammatory cells around the arteries. A: Hematoxylin and eosin staining. B: Elastica van Gieson staining. Original magnification ×100.

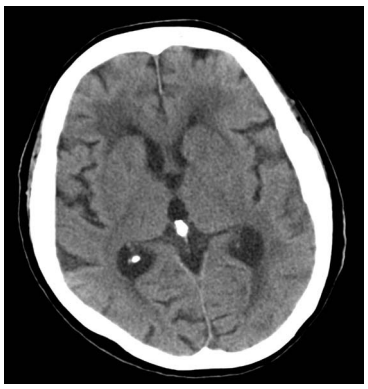

Figure 3 Head computed tomography, which revealed no clear evidence consistent with left hemiparesis.

abdomen and this situation suggests poor prognosis.

In the case of the 70-year-old female patient described here, we needed 6 d before a laparotomy was carried out and 16 d before a definitive diagnosis of polyarteritis nodosa could be made. Reviewing our case retrospectively, there were some findings inconsistent with a typical intestinal infection. First, the findings were remarkable for the presence of renal dysfunction with proteinuria and microscopic hematuria. Second, her abdominal pain and intestinal edema were so severe and resistant to antibiotics. Additionally, in our case, bowel wall thickening was limited to jejunum. We believe that such situation is very unlikely and there is no typical etiology that could explain the symptoms and findings. If we had considered these find-

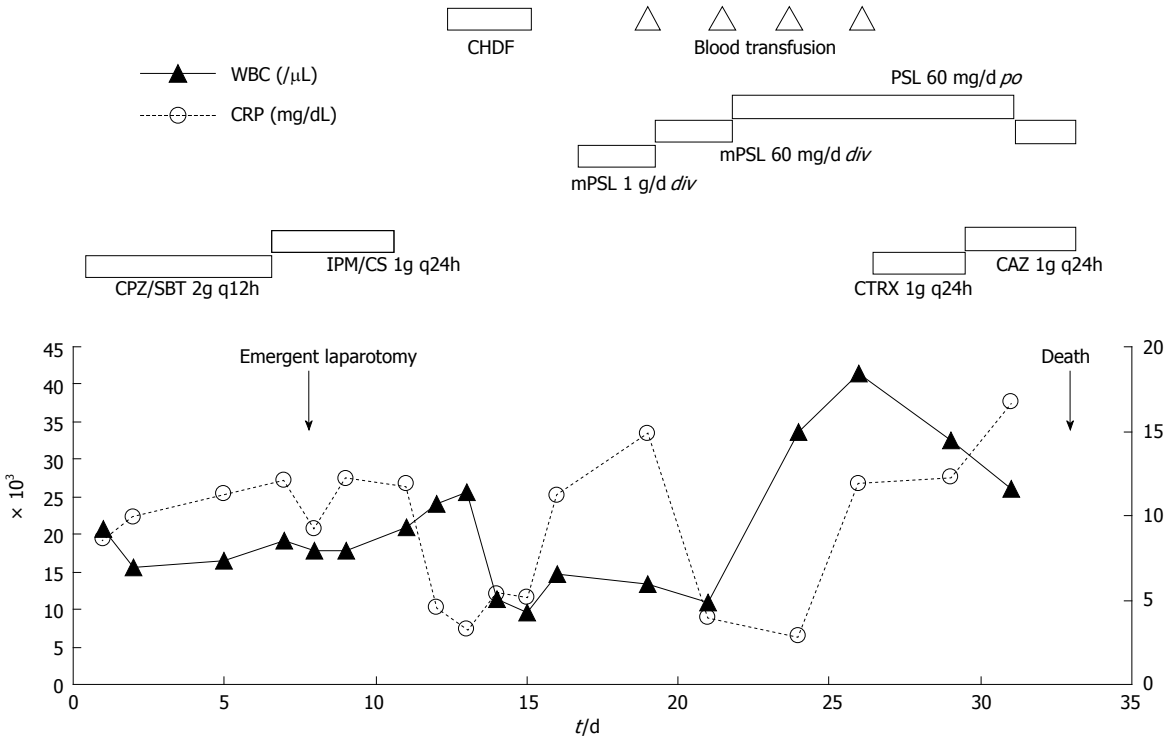

Figure 4 Clinical course of the patient. Cefoperazone/sulbactam (CPZ/SBT) was started at day 1 and switched to imipenem/cilastatin (IPM/CS) at day 7. Ceftriaxone (CTRX) was started at day 26 and switched to ceftazidime (CAZ) at day 29. Steroid pulse therapy [methylprednisolone (mPSL) 1 g/d for 3 d] was started at day 16, and changed to maintenance dose of mPSL (60 mg/d) at day 19, then that was switched to oral steroid (PSL 60 mg/d) at day 22. According to deterioration of general status, steroid was again administered intravenously since day 31. Continuous hemodiafiltration (CHDF) was performed from day 13 to day 15, and intermittent blood transfusion was done at day 19, 21, 23 and 26. WBC: White blood cell; CRP: C-reactive protein.

ings more carefully, we might have suspected vasculitis, or other connective tissue diseases as a differential diagnosis. Nevertheless, since a biopsy remains indispensable for the diagnosis of polyarteritis nodosa and the patient's possible biopsy site was limited to the intestine at that time, we believe that a correct diagnosis was extremely difficult to ascertain in her situation.

Following diagnosis, we performed steroid pulse therapy (methylprednisolone 1000 mg/d for 3 d), followed by a maintenance dose of steroids (60 mg/d for 8 d). Although her inflammatory reaction and renal function initially improved after beginning pulse therapy, the patient subsequently worsened. Although repetitive steroid pulse therapy or another type of immunosuppressive therapy might have been effective in this case, as has been described in the literature[9], we unfortunately did not have sufficient time to start such a treatment before the patient died.

Intestinal ischemia has generally been diagnosed by CT, and bowel wall thickening is one of the most reported findings in that situation[10]. Recently, magnetic resonance imaging (MRI) has begun to be regarded as an alternative modality to detect intestinal ischemia. *In vivo* study with animal model showed that bowel wall thickening was absent from arterial mesenteric ischemia, and bowel wall with reduced thickness resembling sheets of paper is rather observed 2-4 h after the onset, when evaluated by MRI[11]. CT findings in our case are possibly late findings after arterial occlusion, findings caused by reperfusion, or that suggestive of venous infarction. Advances in imaging modality would further clarify the relationship between pathophysiology of intestinal ischemia and radiological findings.

At approximately 12 h before death, a left hemiparesis was observed in our patient, although a head CT revealed no obvious findings. It is difficult to state the cause of the patient's left hemiparesis since she died before a more thorough investigation could be conducted, but it is possible that it stemmed from complications of polyarteritis nodosa, including brain infarction and polyneuropathy[12-14].

In conclusion, we have reported here a case of acute abdomen diagnosed as polyarteritis nodosa based on surgically resected jejunal necrosis. Vasculitis cannot be ruled out as a differential diagnosis in cases of acute abdomen, especially if the patient exhibits atypical findings, such as unexplained renal dysfunction or severe bowel wall thickening limited to small intestine. Additionally, it is worth noting that vasculitis cannot be ruled out even though serum markers, including ANCA, are negative, since there are no definitive diagnostic markers for polyarteritis nodosa.

REFERENCES

1 **Lightfoot RW**, Michel BA, Bloch DA, Hunder GG, Zvaifler NJ, McShane DJ, Arend WP, Calabrese LH, Leavitt RY, Lie JT. The American College of Rheumatology 1990 cri-

teria for the classification of polyarteritis nodosa. *Arthritis Rheum* 1990; **33**: 1088-1093 [PMID: 1975174 DOI: 10.1002/art.1780330805]
2 **Jennette JC**, Falk RJ, Andrassy K, Bacon PA, Churg J, Gross WL, Hagen EC, Hoffman GS, Hunder GG, Kallenberg CG. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 1994; **37**: 187-192 [PMID: 8129773 DOI: 10.1002/art.1780370206]
3 **Mahr A**, Guillevin L, Poissonnet M, Aymé S. Prevalences of polyarteritis nodosa, microscopic polyangiitis, Wegener's granulomatosis, and Churg-Strauss syndrome in a French urban multiethnic population in 2000: a capture-recapture estimate. *Arthritis Rheum* 2004; **51**: 92-99 [PMID: 14872461 DOI: 10.1002/art.20077]
4 **Mohammad AJ**, Jacobsson LT, Mahr AD, Sturfelt G, Segelmark M. Prevalence of Wegener's granulomatosis, microscopic polyangiitis, polyarteritis nodosa and Churg-Strauss syndrome within a defined population in southern Sweden. *Rheumatology* (Oxford) 2007; **46**: 1329-1337 [PMID: 17553910 DOI: 10.1093/rheumatology/kem107]
5 **Guillevin L**, Lhote F, Gayraud M, Cohen P, Jarrousse B, Lortholary O, Thibult N, Casassus P. Prognostic factors in polyarteritis nodosa and Churg-Strauss syndrome. A prospective study in 342 patients. *Medicine* (Baltimore) 1996; **75**: 17-28 [PMID: 8569467 DOI: 10.1097/00005792-199601000-00003]
6 **Levine SM**, Hellmann DB, Stone JH. Gastrointestinal involvement in polyarteritis nodosa (1986-2000): presentation and outcomes in 24 patients. *Am J Med* 2002; **112**: 386-391 [PMID: 11904113 DOI: 10.1016/S0002-9343(01)01131-7]
7 **Pagnoux C**, Mahr A, Cohen P, Guillevin L. Presentation and outcome of gastrointestinal involvement in systemic necrotizing vasculitides: analysis of 62 patients with polyarteritis nodosa, microscopic polyangiitis, Wegener granulomatosis, Churg-Strauss syndrome, or rheumatoid arthritis-associated vasculitis. *Medicine* (Baltimore) 2005; **84**: 115-128 [PMID: 15758841 DOI: 10.1097/01.md.0000158825.87055.0b]
8 **Takada T**, Yoshida H, Tsukada M, Katoh H. A Case of Classic Polyarteritis Nodosa Presenting as Acute Abdomen—A Review of 50 Patients with PN Involving Small Intestine Undergone Laparotomy in Japanese Litetature. *Jpn J Gastroenterol Surg* 2003; **36**: 51-56 [DOI: 10.5833/jjgs.36.51]
9 **Guillevin L**, Lhote F, Cohen P, Jarrousse B, Lortholary O, Généreau T, Léon A, Bussel A. Corticosteroids plus pulse cyclophosphamide and plasma exchanges versus corticosteroids plus pulse cyclophosphamide alone in the treatment of polyarteritis nodosa and Churg-Strauss syndrome patients with factors predicting poor prognosis. A prospective, randomized trial in sixty-two patients. *Arthritis Rheum* 1995; **38**: 1638-1645 [PMID: 7488285 DOI: 10.1002/art.1780381116]
10 **Bartnicke BJ**, Balfe DM. CT appearance of intestinal ischemia and intramural hemorrhage. *Radiol Clin North Am* 1994; **32**: 845-860 [PMID: 8084999]
11 **Berritto D**, Somma F, Landi N, Cavaliere C, Corona M, Russo S, Fulciniti F, Cappabianca S, Rotondo A, Grassi R. Seven-Tesla micro-MRI in early detection of acute arterial ischaemia: evolution of findings in an in vivo rat model. *Radiol Med* 2011; **116**: 829-841 [PMID: 21509555 DOI: 10.1007/s11547-011-0676-7]
12 **Provenzale JM**, Allen NB. Neuroradiologic findings in polyarteritis nodosa. *AJNR Am J Neuroradiol* 1996; **17**: 1119-1126 [PMID: 8791926]
13 **Morelli S**, Perrone C, Paroli M. Recurrent cerebral infarctions in polyarteritis nodosa with circulating antiphospholipid antibodies and mitral valve disease. *Lupus* 1998; **7**: 51-52 [PMID: 9493150 DOI: 10.1191/096120398678919642]
14 **Squintani G**, Ferrari S, Caramaschi P, Cavallaro T, Refatti N, Rizzuto N, Tonin P. Multineuropathy in a patient with HBV infection, polyarteritis nodosa and celiac disease. *Rheumatol Int* 2009; **29**: 579-581 [PMID: 18810449 DOI: 10.1007/s00296-008-0704-7]

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CASE REPORT

Two case reports of gastroendoscopy-associated *Acinetobacter baumannii* bacteremia

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Key words: Endoscopy; *Acinetobacter baumannii*; Bacteremia; Antibiotic prophylaxis

Core tip: After a literature review, we suggest that correct gastroendoscopy technique and skill in drainage procedures, as well as antibiotic prophylaxis, are of paramount importance in minimizing the risk of gastroendoscopy-associated bacteremia. Gastroenterologists should give more attention to gastroendoscopy-related infections, and increased clinical alertness may be the best way to reduce the impact from these types of infections.

Chen CH, Wu SS, Huang CC. Two case reports of gastroendoscopy-associated *Acinetobacter baumannii* bacteremia. *World J Gastroenterol* 2013; 19(18): 2835-2840 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i18/2835.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i18.2835

Abstract

Two cases of gastroendoscopy-associated *Acinetobacter baumannii* (*A. baumannii*) bacteremia were discovered at the study hospital. The first case was a 66-year-old woman who underwent endoscopic retrograde cholangiopancreatography and endoscopic retrograde papillotomy, and then *A. baumannii* bacteremia occurred. The second case was a 70-year-old female who underwent endoscopic retrograde biliary drainage due to obstruction of intra-hepatic ducts, and bacteremia occurred due to polymicrobes (*Escherichia coli*, *viridans streptococcus*, and *A. baumannii*). After a literature review, we suggest that correct gastroendoscopy technique and skill in drainage procedures, as well as antibiotic prophylaxis, are of paramount importance in minimizing the risk of gastroendoscopy-associated bacteremia.

INTRODUCTION

Gastroendoscopy is a commonly used procedure for diagnosis and therapy, such as in endoscopic retrograde cholangiopancreatography (ERCP). Infection is one of the most common morbidity complications of gastroendoscopy. Septic complications of ERCP include ascending cholangitis, liver abscess, acute cholecystitis, infected pancreatic pseudocyst, infection following perforation of a viscus, and, less commonly, endocarditis and endovasculitis[1]. Bacteria can enter the biliary tract by hematogenous or, more frequently, by a retrograde route, and the most common organisms transmitted by ERCP are *Escherichia coli, Klebsiella* species, and *Enterobacter* species[2]. Here we report two interesting cases of gastroendoscopy-associated *Acinetobacter baumannii* (*A. baumannii*)(GEaAb) bacteremia.

CASE REPORT

Case 1

The patient was a 66-year-old woman who visited a gastroenterologist for complaints of right upper quadrant pain and hunger pain. The initial impression was of a gall bladder stone. An ERCP was performed, and it showed a common bile duct of 16.1 mm in diameter and several filling defects in the gall bladder; therefore, an endoscopic retrograde papillotomy (EPT) was executed over the proximal portion of the bile duct, after which a gallstone was removed. The course of the procedure went smoothly. She again began to feel right quadrant pain and fever the next day, so she was admitted for further evaluation and management. At admission, vital sign measurements were: blood pressure, 100/90 mmHg; temperature, 38 ℃; pulse rate, 110 beats/min; and respiratory rate, 20 breaths/min. The patient appeared acutely ill. The abdomen was distended and ovoid. There was radiation pain and tenderness to her back, and abdominal fullness over the right quadrant area (positive Murphy's sign), but no rebounding pain. Admission laboratory results revealed the following: white blood cell count, 12700/mm^3; blood creatinine level, 0.8 mg/dL; serum amylase, 815 IU/L; serum bilirubin, 0.88 mg/dL; aspartate transaminase, 55 U/L; and alanine transaminase, 140 U/L. Abdominal echography revealed peri-pancreatic fluid accumulation. On the first day of admission, the patient was treated with antibiotics (cefazolin 1 g every 8 h plus gentamicin 60 mg every 12 h) and adequate fluid hydration, after initially remaining nil per os (NPO). A blood culture revealed *A. baumannii* on the 4th admission day, and the antibiotic treatment was switched to imipenem-cilastatin 500 mg every 6 h according to the antibiotics susceptibility test. Clinically, the source of *A. baumannii* was from the biliary tract, and it could be related to the previous invasive procedure. Because of persistent fever, an abdominal computed tomography was performed and showed a pancreatic abscess; consequently, an echo-guided aspiration was performed on the 5th admission day. The fever gradually subsided and follow-up laboratory data showed improvement. The total duration of parenteral imipenem-cilastatin usage was 21 d, after which antibiotic therapy was switched to oral levofloxacin 500 mg per os daily. The patient was discharged on the 44th admission day and was followed in the out-patient department (OPD). She has recovered quite well.

Case 2

This patient was a 70-year-old female with liver cirrhosis related to hepatitis C virus infection. At initial presentation, laboratory test results were as follows: serum bilirubin, 0.52 mg/dL; aspartate transaminase, 42 U/L; alfa-fetoprotein, < 20 ng/mL; and hepatitis C virus-antibody titer, positive. The abdominal computed tomography scan showed multiple nodular hypervascular tumor stains in both lobes of the liver, especially in the right lobe. Hepatocellular carcinoma was highly suspected. Transarterial chemo-embolization (TACE) of both sides of the liver was performed, and she was regularly followed in the OPD while she received TACE 6 times over the course of 20 mo. Her follow-up laboratory tests showed a serum bilirubin of 8.6 mg/dL, an aspartate transaminase of 72 U/L, and an alkaline phosphatase of 371 U/L. An abdominal echography exam revealed focal dilated intrahepatic ducts. Hence, an endoscopic retrograde biliary drainage (ERBD) procedure was performed accordingly, at which time a stent (11 Fr) was inserted into the intrahepatic ducts through the common bile duct. She began to feel right quadrant pain and fever three days later, and she was admitted under the impression of cholangitis. At admission, vital signs included a blood pressure of 100/90 mmHg, a temperature of 38 ℃, a pulse rate of 110 beats/min, and a respiratory rate of 20 breaths/min. Murphy's sign was positive. Admission laboratory results revealed the following: white blood cell, 5600/mm^3; serum total bilirubin, 27.65 mg/dL; serum direct bilirubin, 19.2 mg/dL; aspartate transaminase, 60 U/L; and alanine transaminase, 140 U/L. The abdominal echography showed left intra-hepatic duct dilatation. On the first day of admission, the patient was treated with antibiotics (cefazolin 1 g every 8 h plus gentamicin 60 mg every 12 h) and adequate fluid hydration after initially being NPO. Echo-guided percutaneous transhepatic cholangeal drainage was performed. Blood culture revealed polymicrobes (*Escherichia coli*, *viridans streptococcus*, and *A. baumannii*) on the 4th admission day, and treatment was changed to imipenem-cilastatin 500 mg every 6 h according to the antibiotics susceptibility test. The fever gradually subsided. Clinically, the source of *A. baumannii* was from the biliary tract, and it could be related to the previous invasive procedure. Follow-up laboratory data did not, however, seem much improved. This patient expired due to severe hepatic failure with multiple organ failure.

Evidence-based literature review and epidemiological study

After noting those two GEaAb cases in our institute, we conducted an evidence-based literature review (Table 1)[3-7]. Norfleet's study showed that 6% of patients who received upper gastrointestinal endoscopic examinations developed bacteremia, but only one of 447 patients acquired *Acinetobacter* bacteremia[4]. Maulaz's study described that the bacteremia incidence in cirrhotic patients who received variceal ligation was 2.5%, and only one *Acinetobacter lwoffii* infection was disclosed[5]. Only two case reports described post-endoscopic retrograde cholangiopancreatography-associated *Acinetobacter* infection in the United States National Library of Medicine National Institutes of Health[6,7]. Additionally, we performed a retrospective cross-sectional epidemiological study in our institute to identify GEaAb bacteremia cases and elucidate the possible sources of infection for a further five years from the year of identifying these two patients. During this period of five years, we disclosed 45 *A. baumannii* bacteremia cases. Most of them resulted from hospital-

Table 1 Evidence-based literature review for gastroendoscopy-associated *Acinetobacter* bacteremia

Ref.	Country	Evaluation	Risk factors	Microbiology	Treatment	Outcome
Norfleet *et al*[3], 1981	United States	447 patients have been evaluated, of which 6% had bacteremia after upper gastrointestinal endoscopy	Upper gastrointestinal endoscopy	One case with *Acinetobacter sp* infection	NM	NM
Maulaz *et al*[4], 2003	Brazil	The bacteremia incidence in cirrhotic patients submitted to variceal ligation was 2.5%, showing no difference from the control groups	Endoscopic variceal ligation or esophagogast roduodenoscopy only	One case with *Acinetobacter lwoffii* infection	NM	One case with *Acinetobacter lwoffii* infection is survived
Oh *et al*[5], 2007	South Korea	A total of 364 patients who underwent PTC were included in the study	Cholangitis and bacteremia were associated with percutaneous transhepatic biliary drainage and tract dilation, catheter migration and blockage with tract maturation, and bile duct injury with PTC	NM	NM	NM
Lai *et al*[6], 2008	Taiwan	Case report	Endoscopic procedure	Initial, polymicrobes (*Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Enterococcus Faecalis*), then became *Acinetobacter* genomic species 13TU at day 14	Ceftazidime and ampicillin-sulbactam, then intravenous gentamicin and ciprofloxacin (parenteral antibiotics for 4 wk) then followed by oral ciprofloxacin and trimethoprim-sulfamethoxazole (for another 13 d) , antibiotics used for 61 d in total	Survived
de la Tabla Ducasse *et al*[7], 2008	Spain	Case report	Post-endoscopic retrograde cholangiopancreatography	*Acinetobacter ursingii* infection	Cefotaxime	Survived

NM: Not mentioned; PTC: Percutaneous transhepatic cholangioscopy.

acquired pulmonary infection. We focused on biliary tract infections and gastroendoscopy-associated *A. baumannii*, but neither a case of biliary tract infection nor a case of GEaAb were disclosed among hospital-acquired infection patients. So, we excluded those hospital-acquired *A. baumannii* patients, and the results were 19 patients with documented non-hospital-acquired *A. baumannii* bacteremia. We focused on these 19 patients (the demographics and clinical presentations are listed in Table 2). We also performed a pulse-field gel electrophoresis (PFGE) analysis according to Seifert's method[8], and the results are shown in Figure 1. Patients 1, 11 and 14 appeared to have had similar fingerprint patterns according to a dendrogram and PFGE (Figure 1). In the analysis of 5 biliary sepsis cases, risk factors included one patient with common bile duct stone, one with diabetes mellitus, one with cholangiocarcinoma, one with liver cirrhosis, and one with hepatocellular carcinoma. In addition, 3 patients had received invasive diagnostic or therapeutic procedures: one had an EPT, one underwent percutaneous transhepatic cholangeal drainage, and one had ERBD (Table 2). Two of 5 biliary sepsis cases developed *A. baumannii* bacteremia. All of those patients received prophylactic antibiotics before the invasive medical procedures.

DISCUSSION

This is the first serial study and case reports of GEaAb bacteremia in Taiwan. In our study, this infection was seen in one patient after an EPT, and in one patient who underwent ERBD. Both diagnostic and therapeutic gastroendoscopy can lead to bacteremia, and gastroendoscopy-associated infection rates up to 27% have been associated with therapeutic procedures[9-11].

Mechanisms of gastroendoscopy-associated infections

Concerning the mechanisms of gastroendoscopy-associated infections, bacteria can enter the biliary tract by a hematogenous or, more frequently, a retrograde route. Estimates of

Table 2 Demographics and clinical presentations of 19 non-hospital-acquired *Acinetobacter baumannii* bacteremia patients

No.	Age (yr)	Sex	Chief complaint (d)	Previous admission	Initial diagnosis of infection	Underlying disease	Fever/shock	Route of entry[1]	Treatment (d)	Outcome
P1	67	F	RUQ pain (1)	0	Pancreatic abscess	CBD stone	Y/N	Biliary tract[1]	Cefazoline+gentamicin (5), imipenem-cilastatin (12), levofloxacin (8)	S
P2	76	F	Conscious disturbance (3)	3	Liver abscess	DM	Y/N	Biliary tract	Cefmetazole (3), imipenem-cilastatin (7), levofloxacin (7)	S
P3	79	M	SOB (7)	3	Pneumonia	Esophageal cancer	Y/N	Respiratory tract	Co-trimoxazole (4)	S
P4	40	F	SOB (3)	2	Sepsis	Breast cancer	Y/N	Primary[2]	Cefazoline+gentamicin (5), levofloxacin (7)	S
P5	74	M	Deafness (4)	0	Sepsis, sudden deafness	Nil	Y/N	Primary	Cefazoline + gentamicin (1)	S
P6	24	F	Fever, right flank pain (1)	2	Acute pyelonephritis	Pelvic cancer	Y/N	Urinary tract	ampicillin-sulbactam (7), co-trimoxazole (3)	S
P7	76	F	Chest pain (1)	0	Sepsis	AMI	N/Y	Primary	Cefmetazole (4), imipenem-cilastatin (7)	S
P8	80	F	SOB (1)	1	Urinary tract infection	Right renal stone	Y/N	Urinary tract	Cefazoline (5), amikacin (7)	S
P9	82	M	Hematuria (1)	1	Urinary tract infection	Old CVA	Y/N	Urinary tract	Nil	S
P10	1	M	Fever (1)	0	Neonetal infection	Nil	Y/N	Primary	Ampicillin (5)	S
P11	33	F	SOB (3)	1	Sepsis	Cholangiocarcinoma	Y/N	Primary[3]	Cefmetazole (1), imipenem-cilastatin (7)	S
P12	64	M	Abdominal pain (3)	2	Cholangitis	Liver cirrhosis, uremia, AF	N/Y	Biliary tract	Cefazoline (5), gentamicin (14)	E
P13	73	F	Epigastric pain (1)	0	Urosepsis	DM	Y/N	Urinary tract	Imipenem-cilastatin (14)	S
P14	70	M	RUQ pain (1)	2	Cholangitis	HCC, HCV	N/N	Biliary tract[4]	Cefamet (5), imipenem-cilastatin (9)	E
P15	79	M	Loss of consciousness (1)	1	Sepsis	DM	N/N	Primary	Cefazoline (7)	S
P16	79	F	Fever (1)	4	Lung abscess	DKA	Y/Y	Respiratory tract	Cefuroxime (2)	E
P17	80	M	Dysuria (7)	1	Urinary tract infection	DM, urethral stricture HBV	Y/N	Urinary tract	imipenem-cilastatin (14)	S
P18	51	F	Right limb weakness (2)	4	Pneumonia	Chf	N/N	Respiratory tract	Cefazoline (7), co-trimoxazole (7)	S
P19	80	F	Hematuria (3)	1	Urinary tract infection	Left hydronephrosis, right urethral stone	N/N	Urinary tract	Cefazoline (3), urotactin (11)	S

[1]This patient is case one, and she had received the endoscopic papillotomy one day before the episode of *Acinetobacter baumannii* (*A. baumannii*) bacteremia; [2]This patient had suspected hepatocellular carcinoma; [3]This patient had received the percutaneous transhepatic cholangeal drainage three days before the episode of *A. baumannii* bacteremia, and the route of entry of *A. baumannii* could result from biliary tract. He was categorized to primary bacteremia due to lack of typical clinical symptoms and signs of biliary sepsis; [4]This patient is case two, and she had received the endoscopic retrograde biliary drainage one day before the episode of *A. baumannii* bacteremia. RUQ pain: Right upper quadrant pain; SOB: Shortness of breath; AMI: Acute myocardial infarction; TIA: Transient ischemic attack; CBD: Common bile duct; DM: Diabetes mellitus; DKA: Diabetic ketoacidosis; CHF: Congestive heart failure; HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; HCV: Hepatitis C virus; AF: Atrial fibrillation; CVA: Cerebrovascular attack; co-trimoxazole: Sulfamethoxazole-trimethoprim; S: Survived; E: Expired; P: Patient number; F: Female; M: Male; Y: Yes; N: No.

the incidence of clinically significant cholangitis have ranged from 0.4% to more than 10% (mean, 1.4%), depending upon the study population[12]. Entrance into the blood stream is presumably through minor trauma by the endoscope[13]. Another factor influencing the rate of cholangitis is the use of prophylactic antibiotics[14]. Results from these studies were similar to the results in our study.

Organisms associated with gastroendoscopy-associated infections

The most frequent organisms responsible for cholangitis and biliary sepsis are enteric bacteria, such as *Escherichia coli, Klebsiella* species, and *Enterobacter* species[2]. *A. baumannii*, which was reported in this study, is rare, so we performed a molecular epidemiological study. Patients 1, 11 and 14 appeared to have had similar fingerprint patterns according to a dendrogram and PFGE (Figure 1), and those 3 patients had experienced invasive gastrointestinal endoscopic procedures. Although we suspect the relationship, we still cannot prove the causal association between the procedure and infection in this study. We lacked direct microbiological evidence as well as estimates of the infection rate in the gastroendoscopy room. Also, there was no significant evidence of endemic *A. baumannii* infection at Changhua County.

In conclusion, we believe that accurate gastroendoscopy techniques and skill in drainage procedures are of paramount importance for minimizing the risk of GEaAb bacteremia. Antibiotic prophylaxis is also widely considered to be indicated in selected patients. Gastroenterologists should give more attention to gastroendoscopy-related infections,

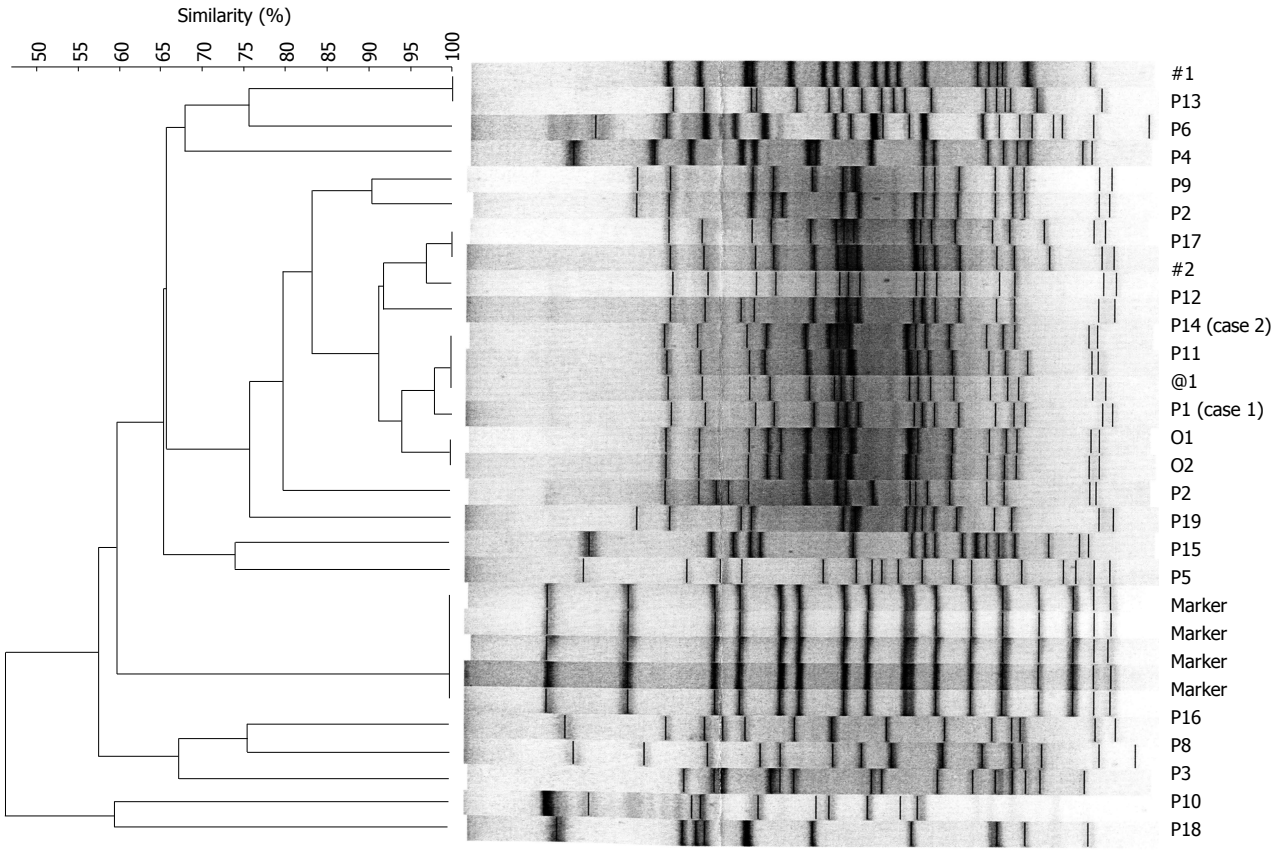

Figure 1 Dendrogram and pulse-field gel electrophoresis patterns of *SgrAI*-digested chromosome DNA of 24 *Acinetobacter baumannii* isolates. #1, #2: Nosocomial *Acinetobacter baumannii* (*A. baumannii*) strain isolated from the same period; @1: Environmental *A. baumannii* strain isolated from the endoscopic room; O1, O2: Outbreak *A. baumannii* strains; P: Clinical *A. baumannii* strains isolated from the numbered patient among 19 non-hospital-acquired *A. baumannii* bacteremia patients.

and increased clinical alertness may be the best way to reduce the impact from these types of infections.

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REFERENCES

1 **Subhani JM**, Kibbler C, Dooley JS. Review article: antibiotic prophylaxis for endoscopic retrograde cholangiopancreatography (ERCP). *Aliment Pharmacol Ther* 1999; **13**: 103-116 [PMID: 10102939 DOI: 10.1046/j.1365-2036.1999.00452.x]

2 **Lin GM**, Lin JC, Chen PJ, Siu LK, Huang LY, Chang FY. Pan-drug resistant Acinetobacter baumannii bacteremia following endoscopic retrograde cholangiopancreatography. *Am J Gastroenterol* 2008; **103**: 498-499 [PMID: 18289224 DOI: 10.1111/j.1572-0241.2007.01646_17.x]

3 **Norfleet RG**, Mitchell PD, Mulholland DD, Philo J. Does bacteremia follow upper gastrointestinal endoscopy? *Am J Gastroenterol* 1981; **76**: 420-422 [PMID: 7337128]

4 **Maulaz EB**, de Mattos AA, Pereira-Lima J, Dietz J. Bacteremia in cirrhotic patients submitted to endoscopic band ligation of esophageal varices. *Arq Gastroenterol* 2003; **40**: 166-172 [PMID: 15029392 DOI: 10.1590/S0004-28032003000300006]

5 **Oh HC**, Lee SK, Lee TY, Kwon S, Lee SS, Seo DW, Kim MH. Analysis of percutaneous transhepatic cholangioscopy-related complications and the risk factors for those complications. *Endoscopy* 2007; **39**: 731-736 [PMID: 17661249 DOI: 10.1055/s-2007-966577]

6 **Lai YH**, Chen TL, Chen CP, Tsai CC. Nosocomial acinetobacter genomic species 13 TU endocarditis following an endoscopic procedure. *Intern Med* 2008; **47**: 799-802 [PMID: 18421203 DOI: 10.2169/internalmedicine.47.0826]

7 **de la Tabla Ducasse VO**, González CM, Sáez-Nieto JA, Gutiérrez F. First case of post-endoscopic retrograde cholangiopancreatography bacteraemia caused by Acinetobacter ursingii in a patient with choledocholithiasis and cholangitis. *J Med Microbiol* 2008; **57**: 1170-1171 [PMID: 18719191 DOI: 10.1099/jmm.0.2008/002253-0]

8 **Seifert H**, Schulze A, Baginski R, Pulverer G. Comparison of four different methods for epidemiologic typing of Acinetobacter baumannii. *J Clin Microbiol* 1994; **32**: 1816-1819 [PMID: 7929781]

9 **Kullman E**, Borch K, Lindström E, Anséhn S, Ihse I, Anderberg B. Bacteremia following diagnostic and therapeutic ERCP. *Gastrointest Endosc* 1992; **38**: 444-449 [PMID: 1511819 DOI: 10.1016/S0016-5107(92)70474-X]

10 **Sauter G**, Grabein B, Huber G, Mannes GA, Ruckdeschel G, Sauerbruch T. Antibiotic prophylaxis of infectious complications with endoscopic retrograde cholangiopancreatography. A randomized controlled study. *Endoscopy* 1990; **22**: 164-167 [PMID: 2209498 DOI: 10.1055/s-2007-1012830]

11 **Niederau C**, Pohlmann U, Lübke H, Thomas L. Prophylactic

antibiotic treatment in therapeutic or complicated diagnostic ERCP: results of a randomized controlled clinical study. *Gastrointest Endosc* 1994; **40**: 533-537 [PMID: 7988813 DOI: 10.1016/S0016-5107(94)70247-0]

12 **Barthet M**, Lesavre N, Desjeux A, Gasmi M, Berthezene P, Berdah S, Viviand X, Grimaud JC. Complications of endoscopic sphincterotomy: results from a single tertiary referral center. *Endoscopy* 2002; **34**: 991-997 [PMID: 12471544 DOI: 10.1055/s-2002-35834]

13 **Mani V**, Cartwright K, Dooley J, Swarbrick E, Fairclough P, Oakley C. Antibiotic prophylaxis in gastrointestinal endoscopy: a report by a Working Party for the British Society of Gastroenterology Endoscopy Committee. *Endoscopy* 1997; **29**: 114-119 [PMID: 9101149 DOI: 10.1055/s-2007-1004085]

14 **Loperfido S**, Angelini G, Benedetti G, Chilovi F, Costan F, De Berardinis F, De Bernardin M, Ederle A, Fina P, Fratton A. Major early complications from diagnostic and therapeutic ERCP: a prospective multicenter study. *Gastrointest Endosc* 1998; **48**: 1-10 [PMID: 9684657 DOI: 10.1016/S0016-5107(98)70121-X]

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In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.00000 35706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

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12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

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13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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