Aims and scope
Yeungnam University Journal of Medicine (Yeungnam Univ J Med, YUJM, eISSN 2384-0293, https://yujm.yu.ac.kr), the official publication of the Yeungnam University College of Medicine, is an international, peer-reviewed, and open access journal in the medical field.

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The first volume was published in December 1984. YUJM is published in English, three times a year (January 31, May 31, and September 30).

YUJM is indexed/tracked/covered by KoreaMed, Korea Citation Index, KoMCI, WPRIM, DOI/CrossRef, and Google Scholar.

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Published on May 31, 2019

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Pharmacologic therapy for nonalcoholic steatohepatitis focusing on pathophysiology

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Introduction
Nonalcoholic fatty liver disease (NAFLD) is a chronic disease in which lipid accumulates in hepatocytes, and hepatocyte ballooning is seen pathologically. The spectrum is wide, ranging from simple steatosis to steatohepatitis (NASH), fibrosis, and even cirrhosis [1]. Lipotoxicity is the main pathophysiology associated with NASH initiation and progression [2]. Even though the main lipid is a form of triglyceride (TG), other lipid metabolites also accumulate. Lipid metabolites, such as free cholesterol (FC) and free fatty acids (FFAs), cause apoptosis via up-regulation of the death receptors, sensitizing them to inflammatory cytokines [2] (Fig. 1). Caspases and the Bcl2 families are involved in the apoptotic pathway [3] (Fig. 2). Even hepatocytes, including Kupffer cells, phagocytize apoptotic cell debris. They release transforming growth factor-β (TGF-β), the main cytokine of fibrosis, which causes the activation of hepatic stellate cells (HSCs) and more accumulation of lipids in the hepatocytes via Smad2/3 [4] (Fig. 3). Therefore, lipotoxicity and apoptosis may be suitable therapeutic targets for the treatment of NASH.

Endotoxin (lipopolysaccharide) activates intrahepatic macrophages (Kupffer cells) via toll like receptor 4 (TLR4), which then release inflammatory cytokines including C-C motif chemokine ligand 2 (CCL2). C-C motif chemokine receptor 2

Keywords: Drug therapy; Nonalcoholic steatohepatitis; Pathophysiology
Fig. 1. Lipotoxicity. Although the major form of lipids is TG, other lipid metabolites also accumulate: FFAs, FC, diacylglycerol, CE, and ceramide. “The quality” rather than “quantity” of lipids may contribute to lipotoxicity. Especially, FC and FFAs are important mediators of lipotoxicity. They up-regulate death receptors, then sensitizing them to TNF-α. They also trigger ER stress, causing mitochondrial dysfunction and apoptosis (adapted from Okazaki I. [Non-alcoholic steatohepatitis]. Cho YK, Eun JR, translators. Paju: Koonja Publishing Inc.; 2018. p. 152 [5], with permission of Koonja Publishing Inc.). TG, triglyceride; FFA, free fatty acid; FC, free cholesterol; CE, cholesterol ester; TNF-α, tumor necrosis factor-α; ER, endoplasmic reticulum; TNF-R1, tumor necrosis factor receptor 1; TRAIL-R, TNF-related apoptosis-inducing ligand receptor; ROS, reactive oxygen species.

Fig. 2. Basic concept of apoptosis. Apoptosis is a key consequence of cell injury. There are two pathways: extrinsic pathway via death-receptors, such as the TNF-α receptor, Fas, and TRAIL; and the intrinsic pathway via ER stress. Both pathways go through the mitochondria. Mitochondrial dysfunction is a key event of apoptosis. Caspases are enzymes associated with the apoptotic process. Caspase 8 is an initiator caspase and caspase 3/7 is an effector caspase. The Bcl2 family is also involved with apoptosis. Bid, Bad, Bim, and Bax/Bak are pro-apoptotic and Bcl2 and Bcl-xL are anti-apoptotic. Caspase 8 occurs cleavage of Bid to tBid, leading to mitochondrial permeabilization and the release of cytochrome C. Released cytochrome C activates caspase 3/7, the effector caspase, resulting in the final apoptotic morphology (adapted from Okazaki I. [Non-alcoholic steatohepatitis]. Cho YK, Eun JR, translators. Paju: Koonja Publishing Inc.; 2018. p. 151 [5], with permission of Koonja Publishing Inc.). TNF-α, tumor necrosis factor-α; TRAIL, TNF-related apoptosis-inducing ligand; ER, endoplasmic reticulum; tBid, truncated Bid; ROS, reactive oxygen species.
CCR2-positive bone marrow (BM)-derived monocytes and HSCs respond to CCL2 and are recruited to the liver [6]. CCR5 is expressed on HSCs and lymphocytes. CCR5 cell infiltration via chemokine-chemokine interaction cause inflammation and fibrosis in the liver [7]. The phase 2b CENTAUR trial was performed to evaluate the efficacy of cenicriviroc (CVC), a dual inhibitor of CCR2/CCR5 [8].

Obesity and insulin resistance are the main risk factor for NASH progression, as well as for cardiovascular disease [9,10]. Hyperglycemia and hyperinsulinemia increase the expression of connective tissue growth factor and activate the type 1 collagen gene in HSCs [9]. Hyperglycemia and hyperinsulinemia have been associated with hepatocellular carcinoma (HCC) in NASH [10]. Insulin sensitizers, such as thiazolidinediones and metformin, are prescribed for the treatment of type 2 diabetes worldwide [11]. Accordingly, they have been investigated as treatments for NASH [12]. Apoptosis signal-regulating kinase 1 (ASK1) is a key enzyme in insulin resistance and inflammation in NASH. The mitochondrial oxidative stress caused by inflammatory cytokines activates c-Jun-N-terminal kinase (JNK) via ASK1 [13]. The activated JNK interrupts insulin action by serine phosphorylation of the insulin receptor substrate 1, not tyrosine phosphorylation [14] (Fig. 4). Selonsertib, an ASK1 inhibitor, improved metabolic parameters in phase 2 clinical trials [14]. Two phase 3 trials are ongoing [13,14].

Cirrhosis is the final event in the fibrotic process. Lysyl oxidase-like 2 (LOXL2) is an enzyme which stabilizes collagen crosslinking. LOXL2 is involved in fibrosis progression, cirrhosis, and even HCC development by directing hepatic progenitor cells toward cholangiocyte differentiation (ductular reaction) [15] (Fig. 5). Simtuzumab, an anti-LOXL2 monoclonal antibody, was tried in clinical trials anticipating an anti-fibrotic effect [16].

Recently, the farnesoid X receptor (FXR), a bile acid synthesis regulator, has gained attention. In the ileum, FXR activates fibroblast growth factor 19 (FGF-19), which increases insulin sensitivity by glucagon-like peptide-1 (GLP-1) activation. Moreover, in the liver, FXR inhibits TG synthesis by inhibiting the sterol regulatory element-binding protein 1c (SREBP-1c) via the short heterodimer partner (SHP), stimulates the β-oxidation of FFAs by peroxisome proliferator-activated receptors (PPAR)-α activation, and improves glucose homeostasis [17] (Fig. 6). Obeticholic acid (OCA), an FXR agonist, has been tested for the treatment of NASH [18].

Although many drugs are under phase 2 or 3 clinical trials, none have been approved by the Food and Drug Administration (FDA).
Fig. 4. ASK1 and insulin resistance. The inflammatory cytokine, TNF-α, produces mitochondrial ROS, which activates ASK1. ASK1 activates JNK, and subsequently causes serine phosphorylation of IRS-1. Normally, the tyrosine phosphorylation of IRS-1 is a key event in the action of insulin. Impaired tyrosine phosphorylation and increased serine phosphorylation of IRS-1 are associated with insulin resistance. ASK1, apoptosis signal-regulating kinase 1; TNF-α, tumor necrosis factor-α; ROS, reactive oxygen species; JNK, c-Jun-NH2-terminal kinases; IRS-1, insulin receptor substrate-1; ROS, reactive oxygen species.

Fig. 5. Lysyl oxidase. The LOX family is composed of four isoforms, LOX and the LOXL1-4. Among them, LOXL2 is a stabilizer of collagen crosslinking. LOXL2 is also expressed in hepatic progenitor cells in fibrotic liver. LOXL2 promotes progenitor cells towards fibrogenic cholangiocytes, suppressing their differentiation into hepatocytes. The ductular reaction characterized by reactive cholangiocytes is associated with hepatocarcinogenesis (adapted from Okazaki I. [Non-alcoholic steatohepatitis]. Cho YK, Eun JR, translators. Paju: Koonja Publishing Inc.; 2018. p. 225 [5], with permission of Koonja Publishing Inc.). LOX, lysyl oxidase; LOXL1-4, LOX-like 1-4; HCC, hepatocellular carcinoma; HPC, hepatic progenitor cell.

https://doi.org/10.12701/yujm.2019.00171
to date. This review will cover the basic principles of pharmacologic therapy in practice, and the important results of phase 2 or 3 clinical drug trials, focusing on pathophysiology in detail.

**Basic principles of pharmacologic therapy**

To treat underlying associated diseases, such as type 2 diabetes, dyslipidemia, and hypertension.

1. **The use of anti-diabetic drugs in cases of co-morbid type 2 diabetes**

   1) Pioglitazone and rosiglitazone

Pioglitazone and rosiglitazone are anti-diabetic drugs of the thiazolidinedione family which stimulate PPAR-γ, decreasing fatty acid migration into the liver and increasing β-oxidation by AMP-activated protein kinase (AMPK) activation [19]. Moreover, PPAR-γ inhibits HSC activation and increases adiponectin levels [19,20]. Randomized controlled trials (RCTs) were conducted based on this background [21]. Insulin resistance and liver enzymes improved during the treatment, but the effect was not sustained after the treatment was stopped. Histologic improvement in steatosis, inflammation, and hepatocellular ballooning was observed. However, fibrosis improvement was not confirmed. Common adverse effects were weight gain and edema. Safety
2. The use of anti-hyperlipidemic agents in cases of dyslipidemia

1) Statins
As mentioned in the introduction section, lipotoxicity is the main pathophysiology of NASH initiation and progression. The altered quality of lipids, rather than their quantity, causes lipotoxicity[2]. Altered lipid metabolites up-regulate death receptors, sensitizing them to inflammatory cytokines. They also cause endoplasmic reticulum (ER) stress. Extrinsic and intrinsic stimuli cause mitochondrial oxidative stress, then finally, apoptosis. Moreover, FC accumulation in the HSCs increases TLR4, then activates TGF-β via bone morphogenic protein and activin membrane-bound inhibitor inhibition, which causes a vicious cycle of FC accumulation[30].

Statins have lipid-lowering effects as HMG-CoA reductase inhibitors. They also have anti-oxidant and anti-inflammatory effects[2]. Atorvastatin has been reported to decrease mitochondrial FC and increase glutathione levels[2]. These agents should be considered for the prevention of cardiovascular diseases in NAFLD patients with hypercholesterolemia. Several studies have suggested that statins may improve liver enzymes and histology in patients with NASH. However, no RCTs with histological endpoints have been conducted. Until RCTs with histological endpoints are undertaken, statins are not recommended for the treatment of NASH without dyslipidemia[23].

2) Omega-3 fatty acids
N-6 fatty acids have inflammatory, and n-3 fatty acids have anti-inflammatory action. Moreover, an increased n-6/n-3 fatty acids ratio increased the HCC risk in a NASH mouse model[31]. Omega-3 fatty acids showed improvement in hepatic steatosis, insulin sensitivity, oxidative stress, and anti-inflammatory action in an animal model[32]. Omega-3 fatty acids are currently approved in the United States to treat hypertriglyceridemia[23]. Two large studies failed to show therapeutic benefit in patients with NAFLD/NASH[33,34]. Therefore, they are not recommended for the treatment of NAFLD without hypertriglyceridemia[23].

3) Fibrates
Fibrates, such as bezafibrate or fenofibrate, are extensively used for the treatment of hypertriglyceridemia. But there is little data on NAFLD/NASH.

4) Ezetimibe
Ezetimibe inhibits the intestinal absorption of luminal cholesterol by binding to the Niemann-Pick C1-like 1 transporter in the membrane of the enterocyte brush border. In an animal model,
it reduced cholesterol absorption up to 15 to 20% and hepatic fat content, and improved insulin resistance [35]. But the MOZART RCT trial failed to demonstrate reductions in liver fat contents or an improvement in the liver histology of NASH patients [36].

3. Angiotensin receptor blockers in cases of co-morbid hypertension
Angiotensin II type 1 receptors are expressed on activated HSCs. Therefore, angiotensin receptor blocker (ARBs) may have anti-fibrotic effects in NASH. In an animal study, olmesartan improved fibrosis in NASH [37]. In a study of 54 NASH patients, telmisartan improved insulin resistance and NASH scores compared to valsartan [38]. A losartan study in NASH patients failed because of slow recruitment of patients due to the already-widespread use of ARB in NASH patients [39].

New drugs under clinical trial
OCA, selonsertib, CVC, and elafibranor are in phase 3 trials. Emricasan, aramchol, simtuzumab, NGM282, and BMS-986036 trials have been completed or are in phase 2 trials [40].

1. Farnesoid X receptor agonist: obeticholic acid
OCA is a first-in-class selective FXR agonist. Its mechanism of action scheme is shown in Fig. 6. In an animal model, OCA improved insulin sensitivity, glucose and lipid metabolism, and showed anti-inflammatory and anti-fibrotic effects in hepatic, renal, and intestinal tissues [41]. Two major phase 2 clinical trials were conducted to evaluate the safety and efficacy of OCA in biopsy-proven NASH patients [41,42]. In the FLINT phase 2b trial (n=283), 45% of the patients in the 72-week OCA group achieved the primary outcome (a decrease in NAFLD activity score by ≥2 points without worsening of fibrosis) compared to 21% in the placebo group (p=0.0002). Fibrosis improvement was observed in 22% of the patients in the OCA group compared to 13% in the placebo group (p=0.08). The main adverse events of OCA were increased low-density lipoprotein (LDL) cholesterol, decreased high-density lipoprotein cholesterol and pruritus (23%) [42]. The CONTROL trial (NCT02633956) combining statins is ongoing. Two international phase 3 trials, RENERATE (NCT02548351) and REVERSE (NCT03439254) are now ongoing [41].

2. ASK1 inhibitor: selonsertib
ASK1 activation by mitochondrial oxidative stress, then JNK activation, is an important process in insulin resistance and inflammation [43]. The schematic mechanism of action is shown in Fig. 4. Selonsertib, an ASK1 inhibitor, significantly improved, not only metabolic parameters but also histologic parameters, such as hepatic steatosis, inflammation, and fibrosis [14]. In a 24-week clinical trial with or without simtuzumab, 43% (13/30) of the patients in the 18 mg selonsertib group showed reduced fibrosis (≥one stage) compared with 30% (8/27) in the 6 mg selonsertib group and 20% (2/10) in simtuzumab-alone group [14]. Two phase 3 clinical trials, STELLAR-3 (NCT03053050) and STELLAR-4 (NCT03053063), are now ongoing [40]. Common adverse effects are headache, nausea, fatigue, and upper abdominal pain [14].

3. CCR2/CCR5 dual inhibitor: cenicriviroc
The role of CCR2 and CCR5 for NASH progression are described in the introduction section. CVC is a potent inhibitor of CCR2/CCR5. In the phase 2b CENTAUR trial (n=289), CVC did not meet the primary endpoint (≥2-point NAS improvement or NASH resolution) but showed ≥ one stage fibrosis improvement after 1 year of treatment (20% vs. 10% in the placebo group, p=0.023). In contrast, CVC did not change body weight, aminotransferase levels, or insulin resistance. It was safe and tolerable, especially in terms of infection concerns [8]. The large phase 3 AURORA trial (NCT03028740) was initiated based on the efficacy and safety data of the CENTAUR trial [7,40]. About 2,000 patients were randomized 2:1 (CVC 150 mg or placebo) to evaluate liver fibrosis improvement. They will undergo three consecutive liver biopsies (baseline, after 1 and 5 years). The trial will end in 2019. FDA approval might be determined by the outcome of the trial [40].

4. PPAR α/δ agonist: elafibranor (GFT505)
PPARs are composed of three isoforms: α, β/δ, and γ. PPARs are expressed in many tissues but differently distributed between the isoforms. For example, the α isoform is mainly in the liver and skeletal muscles, while the δ isoform is found in all tissues. PPARs participate in fatty acid oxidation and energy balance. Elafibranor (GFT505), a dual PPAR α/δ agonist, improved plasma lipids and glucose homeostasis, insulin resistance, and reduced liver inflammatory markers [44]. The phase 2b GOLDEN-505 trial (NCT0164849) was conducted to evaluate the safety and efficacy of elafibranor. A total of 276 NASH patients without cirrhosis were randomized to three groups (120 mg, 80 mg, and placebo). The primary endpoint (NASH reversal without progression of fibrosis at 52 weeks) was not met but post-hoc analysis based on a modified definition of response (disappearance of ballooning with the disappearance or mild persistence of lobular inflammation and no worsening of fibrosis), showed significant superiority in
the 120 mg group. Liver enzymes, LDL-cholesterol, HbA1c, and inflammatory markers were significantly reduced in the 120 mg elafibranor group. It was tolerated and safe, even though it caused a mild, reversible increase in serum creatinine [44]. The phase 3 RESOLVE-IT trial (NCT02704403) began in 2016 with the goal of recruiting 200 patients. Active recruitment will be completed in Dec 2021 [40].

5. Pan-caspase inhibitor: emricasan
Caspases are key enzymes in the apoptotic pathway. The detailed mechanism of action is shown in Fig. 2. Emricasan is a pan-caspase inhibitor which blocks apoptosis. In phase 2 clinical trial (NCT02077374), 28-day emricasan therapy decreased ALT, cytokeratin 18, and caspase 3/7 significantly. It was safe and well-tolerated [45]. In the clinical study of 23 patients with compensated cirrhosis, 28-day emricasan treatment decreased portal pressure in a subgroup of patients with severe portal hypertension (hepatic venous pressure gradient ≥12 mmHg) [46].

6. SCD1 modulator: aramchol
Stearoyl-coenzyme A desaturase 1 (SCD1) is a key enzyme which converts saturated fatty acid (SFA) to monounsaturated fatty acid (MUFA). SCD1 expression results in MUFA formation. In contrast, its deficiency results in SFA accumulation. Over-accumulation of SFA may result in ER stress and apoptosis [2]. Aramchol, a conjugate of cholic acid and arachidonic acid, is an inhibitor of SCD1. In a phase 2 clinical trial (n=58, NCT01094158), 300 mg aramchol treatment for 3 months decreased liver fat content and increased adiponectin levels significantly. Aramchol was safe and tolerable at a 300 mg dose [47]. Further data are lacking.

7. Monoclonal LOXL2 antibody: simtuzumab (GS-6624)
The lysyl oxidase (LOX) family is composed of four isoforms, LOX and the LOXL1-4. Among them, LOXL2 is a key contributor to collagen crosslinking stabilization. Moreover, LOX2 promotes hepatic progenitor cells towards the cholangiocyte lineage, while suppressing their differentiation into hepatocytes (Fig. 5) [15]. Theoretically, blocking LOX2 activity attenuates collagen crosslinking and fibrosis, and promotes liver regeneration. Based on this background, a phase 2b clinical trial of simtuzumab, an anti-LOXL2 monoclonal antibody, was conducted. Unfortunately, simtuzumab was ineffective in decreasing collagen content or the hepatic venous pressure gradient (NCT01672879) [16].

8. Fibroblast growth factor 19 agonist; NGM282
NGM282 is a variant of FGF-19, which reduces steatosis and lipotoxicity. In a phase 2 trial of 82 biopsy-proven NASH patients, 79% of the treatment group achieved the primary endpoint (≥5% reduction in absolute liver fat content by magnetic resonance imaging-proton density fat fraction after 12 weeks of treatment) compared to 7% in the placebo group (NCT02443116). ALT level decreased and LDL cholesterol was increased in the treatment group [48].

9. FGF-21 pegylated analogue; BMS-986036
BMS-986036 (Pegbelfermin) is a pegylated analogue of FGF-21. BMS-986036 decreased hepatic steatosis, NAFLD activity score (NAS) and fibrosis in a mouse NASH model, and improved insulin sensitivity, lipid profiles and fibrotic markers in obese diabetic patients. In a phase 2a trial of 75 obese biopsy-proven NASH patients, pegbelfermin achieved the primary endpoint and was tolerated during 16 weeks of treatment. The absolute hepatic fat fraction decreased 6.8% in the group who received a daily injection of 10 mg pegbelfermin, 5.2% in the 20mg weekly injection group, and 1.3% in the placebo injection group (p=0.0004, 0.008, respectively). Pegbelfermin had beneficial effects on adiponectin levels, lipid profiles, aminotransferase levels, serum pro-C3, and liver stiffness [49].

Conclusion
The pathophysiology of NASH progression is complex. Therefore, one drug targeting a single pathway may not be effective. That is the reason why many drugs failed in clinical trials. Although several drugs succeeded in phase 2 trials and moved on to phase 3, the efficacies were modest. Therefore, further research may be focused on combined therapy with two or more drugs covering different mechanisms of action.

Conflicts of interest
No potential conflicts of interest relevant to this article was reported.

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https://doi.org/10.12701/yujm.2019.00171


Trends in the study on medical education over the last 10 years, based on paper titles

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Introduction

Research subjects in medical education are incredibly diverse and have changed with the times. In the past 10 years, the annual conferences held by the Korean Society of Medical Education (KSME) had the following themes: In 2009 (the 25th Annual Conference), the main theme was “From Scholarly Medicine to Professional Competency”; “Medical Humanities” in 2010; “Medical Education: Reflection and Reform” in 2011; “Outcome-Based Medical Education” in 2012; “Challenges for Future Medical Education” in 2013; “Culture and Environment: Medical Education in Korea” in 2014; “Professoring” in 2015; “Students in Medical Education” in 2016; “Future Medical Education and Artificial Intelligence” in 2017; and “A New Horizon for the Doctor Training System in Medical Education” in 2018. Each year, the KSME has decided upon a main theme to match the changes in the medical education and social environments [1].

Many medical education associations exist worldwide, such as the Association of American Medical Colleges (AAMC), the Association of Medical Education in Europe (AMEE), the Association of Medical Schools in Europe, the Asian Medical Education Association, the General Medical Council in the United Kingdom, the Canadian Association for Medical Education, and the Sociedad Española de Educación Médica [2]. These organizations also publish medical education journals, such as Academic Medicine, the journal of the AAMC; BMC Medical Education;
In Korea, the KSME publishes the Korean Journal of Medical Education (KJME). Another medical education journal in Korea is the Korean Medical Education Review (KMER), published by the Department of Medical Education at Yonsei University.

This study aimed to compare and analyze trends in medical education research using the words from research paper titles.

### Medical education journals

For the purpose of this paper, two of the world’s most prestigious journals — and especially in the United States and Europe — were chosen over global studies for an analysis: Academic Medicine, the journal of the AAMC, and Medical Teacher, the journal of the AMEE. The KJME and KMER, two Korean medical education journals, were chosen to examine studies in Korea.

From 2009 to 2018 (Table 1), the KJME published 410 papers, or an average of 41 papers per year, ranging from 30 to 50 per year [3]. The KMER published 205 papers, or an average of 20.5 papers a year, ranging from 9 to 30 per year [4]. Academic Medicine published 4,608 papers, or approximately 460 papers published annually, ranging from 376 to 563 per year [5]. Medical Teacher published 3,745 papers and an average of 374.5 papers a year, ranging from 325 to 442 per year [6]. Academic Medicine and Medical Teacher publish nearly 10 to 20 times more papers than those published in Korean journals.

### Frequencies of words used in the titles

A word cloud (RStudio; RStudio, Boston, MA, USA) uses large-sized words to represent words’ increasing frequency [7]. When examining the frequencies of words in titles, the larger words in the word cloud primarily involve such connecting words as “and,” “of,” “for,” “to,” “in,” “the,” and “on,” among others (data not shown); therefore, these words occur frequently. After removing these connections, the words “medical,” “student,” “health,” and “education” appeared as larger words. High-frequency words in titles include “medical,” “student,” “education,” and “learning” in the KJME; “medical,” “educational,” “study,” and “development” in the KMER; “medical,” “education,” “health,” and “medicine” in Academic Medicine; and “medical,” “education,” “student,” and “learning” in Medical Teacher (Fig. 1).

The word “medical” appeared in 248 of 410 papers (60.49%) in the KJME, 117 of 205 papers (57.07%) in the KMER, 1,405 of 4,608 (30.49%) papers in Academic Medicine, and 1,602 of 3,745 papers (42.78%) in Medical Teacher. The word “education” appeared 21.46%, 41.46%, 17.17%, and 21.66% of the time in the KJME, KMER, Academic Medicine, and Medical Teacher, respectively. The word “student” occurred 38.29% of the time in the KJME, 21.95% in the KMER, 11.00% in Academic Medicine, and 25.58% in Medical Teacher. These words appeared in the titles of the medical education journals because medical schools direct students’ medical education (Tables 2, 3).

Most professors and staff in medical schools want to know how to teach students well, how students can learn well, the best way to administer a clinical education, how to improve students’ clinical performance, what we must teach students to improve their clinical skill, how to rate students to check their level of education, and how to make beneficial programs for the students, among others. Therefore, the frequency of use for such words as “teaching,” “learning,” “clinical,” “evaluation,” “skill,” “curriculum,” and “program” were investigated (Tables 2, 3). The frequencies in the KJME were as follows: “learning” (13.66%), “clinical” (13.66%), “skill” (8.05%), “program” (7.07%), “evaluation” (5.85%), “teaching” (5.61%), and “curriculum” (3.41%). The frequencies of use in the KMER were “learning” (12.20%), “clinical” (7.80%), “skill” (2.44%), “program” (9.76%), “evaluation” (1.95%), “teaching” (7.80%), and “curriculum” (9.27%). The frequencies in Academic Medicine were “learning” (4.41%), “clinical” (6.47%), “skill” (2.04%), “program” (6.32%),

### Table 1. Number of papers in each journal of medical education during last 10 years

<table>
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KJME, Korean Journal of Medical Education; KMER, Korean Medical Education Review.
“evaluation” (1.80%), “teaching” (3.49%), and “curriculum” (1.50%). The frequencies in Medical Teacher were “learning” (11.56%), “clinical” (10.44%), “skill” (5.10%), “program” (5.18%), “evaluation” (2.91%), “teaching” (8.68%), and “curriculum” (4.97%). These results indicate that Korean journals and Medical Teacher have a relatively high proportion of the terms “clinical” and “learning” (7.80% to 13.66%), but Academic Medicine has a relatively low proportion (6.47% and 4.41%, respectively). Therefore, each journal has a slightly different direction to its pursuits.

Changes in several research topics over 10 years

According to Kim et al. [8], problem-based learning (PBL) was introduced to Korean medical schools through a research paper that described the Harvard Medical School's curriculum in 1992. Further, a PBL study group was organized under the KSME in 1995, with 15 medical schools applying PBL in their curriculum by 1999.

Team-based learning (TBL) is a widely used educational
method that has been verified in medical education worldwide. It includes such factors as self-directed learning, teamwork, interpersonal communication, peer learning, and feedback, all of which healthcare professionals require. Significant evidence also exists that this method positively impacts academic achievement (e.g., summative assessments). Students prefer TBL over passive lectures or other types of small-group learning [9].

An educational program should be developed to both determine and outline what students can accomplish by the end of the course, and to note what they must learn to achieve target levels of knowledge and performance. Evaluations must then occur to ensure the program’s successful implementation [9].

Medical students are highly aware of this academic burden; therefore, it is necessary to explore students’ stressful experiences and adaptive endeavors as they progress in their degree programs and the curricula change [10].

Digital devices—such as computers, smartphones, and tablets—are replacing such items as traditional printed books and paper notebooks. Interactive e-learning lectures are also used to supplement traditional lectures’ shortcomings in many educational areas. Smartphones have become essential due to the development of mobile devices and wireless communication technologies. Interest has also recently increased in the 4th Industrial Revolution and artificial intelligence (AI), as reflected in the main theme of the KSME’s 2017 annual conference, “Future Medical Education and Artificial Intelligence.”

Research on these various topics—such as PBL, TBL, program evaluations, burnout, e-learning, and digital—were investigated over a 10-year period, from 2009 to 2018 (Fig. 2).

The concept of PBL was mentioned in 27 papers from the

Table 2. The word frequencies in the title of papers in the KJME and the KMER during year 2009 to 2018

<table>
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<th>%</th>
<th>Term</th>
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</table>

KJME, Korean Journal of Medical Education; KMER, Korean Medical Education Review.

Table 3. The word frequencies in the title of papers in the Academic Medicine and the Medical Teacher during year 2009 to 2018

<table>
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<tr>
<th>Term</th>
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<th>Term</th>
<th>Medical Teacher No.</th>
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https://doi.org/10.12701/yujm.2019.00206
Fig. 2. The number of papers showing MeSH Major Topics, such as “problem-based learning” (A), “program evaluation” (B), and “burnout” (C), and/or such topic words in titles of papers as “team-based learning” (D), “e-learning” (E), and “digital” (F) in the KJME, KMER, Academic Medicine, and Medical Teacher from 2009 to 2018. MeSH, Medical Subject Headings; KJME, Korean Journal of Medical Education; KMER, Korean Medical Education Review.

KJME, 1 from the KMER, 55 from Academic Medicine, and 159 from Medical Teacher. The KJME, Academic Medicine, and Medical Teacher covered PBL almost annually, but the KMER did not. Medical Teacher covered PBL more than Academic Medicine, although the former published fewer papers than the latter. In 2012, Medical Teacher covered PBL in 32 papers, but Academic Medicine discussed PBL in only 1 paper.

While TBL was mentioned in 10 papers from the KJME, 22 from Academic Medicine, and 38 from Medical Teacher, the KMER did not cover TBL. Medical Teacher also covered TBL.
more than Academic Medicine. In 2005, Academic Medicine first covered TBL, and Medical Teacher first covered PBL in 2006. Medical Teacher then covered PBL through 2 to 8 papers every year.

Program evaluations were mentioned in 15 papers from the KJME, 2 from the KMER, 34 from Academic Medicine, and 57 from Medical Teacher. The KJME, Academic Medicine, and Medical Teacher covered program evaluations nearly annually, but the KMER covered only two papers in 2018. Medical Teacher also covered program evaluations annually, ranging from 2 to 13 papers, although not in 2016.

Burnout was mentioned in 5 papers from the KJME, 44 from Academic Medicine, and 16 from Medical Teacher, while the KMER did not cover burnout. The KJME covered burnout the most recently, over 4 years from 2014 to 2017. Academic Medicine covered burnout more than Medical Teacher and did so annually, ranging from 2 to 9 papers.

e-Learning was mentioned often in Medical Teacher, but Academic Medicine and the KJME covered this topic only 6 and 1 times, respectively, while the KMER did not cover it. Medical Teacher covered 59 papers, with every year including 1 to 12 papers.

Digital was mentioned in 2 papers from the KJME, in 2011 and 2017; 1 from the KMER in 2014; 25 from Academic Medicine, and 40 from Medical Teacher. While Academic Medicine covered the digital topic every year, with 1 to 4 papers published each year—except in 2015—Medical Teacher first covered the digital topic annually, ranging from 1 to 8 papers published.

These results illustrate that Medical Teacher seems to primarily discuss teaching and learning methodologies, and Academic Medicine evenly addresses all areas of medical education. As the KJME and KMER publish 1/10 to 1/20 of the number of papers compared to Academic Medicine and Medical Teacher, it is difficult to study all subjects. However, it is anticipated that new subjects—such as AI in medical education—will be researched in the near future.

**Conflicts of interest**

No potential conflicts of interest relevant to this article was reported.

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**References**


m = Acad+Med%5BJour%5D+AND+2009-2018%5Bp-dat%5D&cmd=detailssearch
Endoscopic features aiding the diagnosis of gastric mucosa-associated lymphoid tissue lymphoma

Byung Sam Park, Si Hyung Lee
Division of Gastroenterology and Hepatology, Department of Internal Medicine, Yeungnam University College of Medicine, Daegu, Korea

The incidence of gastric mucosa-associated lymphoid tissue (MALT) lymphoma is increasing worldwide, but the diagnosis is difficult. Most patients are asymptomatic or complain of nonspecific gastrointestinal symptoms. As the endoscopic features of gastric MALT lymphoma are variable and nonspecific, the possibility of this condition may be overlooked during esophagogastroduodenoscopy, and it remain undiagnosed. Therefore, this condition needs to be considered when an abnormal mucosa is observed during this procedure. Biopsy performed during endoscopy is the primary diagnostic test, but false negative results are possible; large numbers of samples should be collected from both normal and abnormal mucosa. Endoscopic ultrasonography is useful to assess the depth of invasion and to predict the treatment response. After treatment, follow-up tests are required every 3 months until complete remission is achieved, and annually thereafter. Early diagnosis of gastric MALT lymphoma is difficult, and its diagnosis and follow-up require wide experience and competent endoscopic technique.

Keywords: Diagnosis; Follow-up studies; Gastrointestinal endoscopy; Marginal zone B-cell lymphoma; Stomach neoplasms

Introduction

Primary gastric lymphoma is the most common extranodal lymphoma, accounting for 2–7% of gastric malignancies [1]. Histologically, mucosal-associated lymphoid tissue (MALT) lymphoma accounts for approximately 40% of primary gastric lymphoma, and diffuse large B-cell lymphoma accounts for most of the remainder [2,3]. MALT lymphoma accounts for 5–8% of B-cell lymphomas and may occur in the entire digestive system, but most commonly in the upper gastrointestinal tract [4,5]. MALT lymphoma was first reported by Isaacson and Wright in 1983 [6] and classified as a B-marginal zone lymphoma and extranodal type in the Revised European American Lymphoma classification in 1994. In 2008, the World Health Organization classification showed extranodal marginal zone B-cell lymphoma of MALT.

Helicobacter pylori (H. pylori) infection is closely related to the development of gastric MALT lymphoma and is associated with 90% of gastric MALT lymphoma [7,8]. H. pylori eradication therapy can induce complete remission of low-grade gastric MALT lymphoma in 80% of patients, and favorable long-term prognosis had been shown [9-11]. However, most patients with gastric MALT lymphoma are asymptomatic or complain of nonspecific gastrointestinal symptoms, and the endoscopic features of gastric MALT lymphoma are variable and nonspecific, making it difficult to distinguish from gastritis, erosion, benign gastric ulcer, and gastric cancer [12]. In addition, the time until remission of gastric MALT lymphoma after H. pylori eradication therapy may be long [13], and recognition of remission is difficult because of atrophied and discolored mucosa. Therefore, diagnosis and follow-up of gastric MALT lymphoma require wide experience and competent
endoscopic technique. Here, we describe the diagnosis and follow-up of gastric MALT lymphoma with emphasis on endoscopic findings.

### Diagnosis of gastric MALT lymphoma

#### 1. Esophagogastroduodenoscopy

The endoscopic features of gastric MALT lymphoma can be classified into exophytic, ulceroinfiltrative, and superficial types; ulceroinfiltrative type is the most common, accounting for approximately 40–50% of all cases [14-17]. Yokoi et al. categorized the superficial types of gastric MALT lymphoma into IIc-like, submucosal tumor, multiple erosion, cobblestone mucosa, partial fold-thickening, and discoloration types [18]. Gastric MALT lymphoma may be missed during esophagogastroduodenoscopy (EGD) because its endoscopic features are variable and nonspecific. Therefore, this condition needs to be considered when abnormal mucosa is observed during this procedure (Fig. 1).

Endoscopic biopsy using forcep and histopathologic examination are the most basic tests for diagnosis of gastric MALT lymphoma. However, false-negative results in the histologic examination may be possible because the tumor cell of gastric MALT lymphoma originates from the deep mucosa or submucosa and grows without destroying the foveolar gland, which is the basic structure of the mucosal surface [19,20]. Therefore, repeated EGD and endoscopic biopsy may be needed for accurate diagnosis of gastric MALT lymphoma. Furthermore, because gastric MALT lymphoma may have a multifocal distribution of tumors and high-
grade gastric MALT lymphoma may be present together, multiple endoscopic biopsies should be performed in the normal mucosa of the antrum, greater and lesser curvatures of the body, and fundus, as well as the abnormal mucosa of at least two tissues in each part [21]. More invasive tissue biopsy such as endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) may be required if the diagnosis is not confirmed by endoscopic biopsy [22].

Lymphoepithelial lesion is characteristic feature of gastric MALT lymphoma. Wotherspoon et al. [23] proposed a histological classification system to differentiate normal gastric mucosa and gastric MALT lymphoma (Table 1). Gastric MALT lymphoma can be diagnosed if a histopathologic change corresponding to a score of 5 is observed. If the score is 3 or 4, diagnosis is confirmed based on the monoclonality of B-cells in polymerase chain reaction (PCR) [24]. In addition to morphological evaluation of the tissues, immunohistochemical staining of cluster of differentiation (CD) 20, CD79a, CD43, and more can be performed to diagnose gastric MALT lymphoma. A balanced translocation, t(11;18) (q21;q21), is found in 25–30% of gastric MALT lymphoma cases [25], and it was shown to be associated with a low response to H. pylori eradication therapy and poor prognosis [26,27]. Moreover, t(11;18) (q21;q21) assessed by fluorescence in situ hybridization or API2/MALT1 fusion gene, which is the result of chromosomal translocation, detected by reverse transcriptase PCR, can identify the patients who are expected to have low therapeutic response to H. pylori eradication therapy.

Confirming H. pylori infection in gastric MALT lymphoma is important because the presence of H. pylori affects treatment strategy and response. H. pylori infection must be detected by rapid urease test, histological examination, culture test, urea breath test, and stool antigen test [28], and the presence of H. pylori should be confirmed during the histopathologic evaluation for the diagnosis of gastric MALT lymphoma. Because hematoxylin and eosin staining has low sensitivity for H. pylori diagnosis and has difficulty in detecting non-spiral and spherical H. pylori, special stainings, such as Giemsa, Warthin–Starry, and Alcian blue, or immunostaining for H. pylori, which is the most sensitive and specific, may be required to confirm H. pylori infection [29,30].

Recently, several studies have been reported to improve the diagnosis of gastric MALT lymphoma by using imaging-enhanced endoscopy, such as narrow-band imaging or linked color imaging [31,32].

2. Endoscopic ultrasonography
Endoscopic ultrasonography (EUS), which has sensitivity of 89%, a specificity of 97%, and a total accuracy of 97%, is the most accurate test to assess the depth of tumor invasion of gastric MALT lymphoma [33,34]. EUS is also useful for evaluating local lymph node metastasis. EUS findings of gastric MALT lymphoma can be classified into four types: superficially spreading, diffusely infiltrating, mass forming, and mixed. Superficially spreading and diffusely infiltrating types are seen in low-grade gastric MALT lymphoma, and tumor invasion into the whole gastric wall and lymph node enlargement are characteristics of high-grade gastric MALT lymphoma [35].

EUS can be used to diagnose gastric MALT lymphoma. A thickened gastric wall of 6–12 mm in the EUS can predict gastric MALT lymphoma, and EUS–guided fine needle aspiration (EUS–FNA) can provide diagnostic information by securing false negatives that may occur in endoscopic biopsy [36,37].

EUS can help predict the treatment response of gastric MALT lymphoma. When the gastric MALT lymphoma infiltrates into the submucosal layer in EUS, the complete remission rate for H. pylori eradication therapy is lower than that for the mucosal layer. When the tumor invades the muscle layer, complete remission was not achieved by H. pylori eradication therapy [38,39] (Fig. 2).

After the treatment of gastric MALT lymphoma, therapeutic response or relapse after the remission could be evaluated by tracking the changes in the gastric wall thickness by EUS [40]. However, even when remission is achieved, normalization of the thickened gastric wall occurs slowly, and EUS may show normal findings even if the tumor remains histologically [41]. Nevertheless, if thickened stomach wall is observed on EUS even

| Table 1. Wotherspoon histologic scoring system for diagnosis of MALT lymphoma [23] |
|---|---|
| Score | Diagnosis | Histologic features |
| 0 | Normal | Scattered plasma cells in lamina propria. No lymphoid follicles. |
| 1 | Chronic active gastritis | Small clusters of lymphocytes in lamina propria. No lymphoid follicles. No lymphoepithelial lesions. |
| 2 | Chronic active gastritis with florid lymphoid follicle formation | Prominent lymphoid follicles with surrounding mantle zone and plasma cells. No lymphoepithelial lesions. |
| 3 | Suspicious lymphoid infiltrate, favor reactive | Lymphoid follicles surrounded by small lymphocytes that infiltrate diffusely in lamina propria and occasionally into epithelium. |
| 4 | Suspicious lymphoid infiltrate, favor lymphoma | Lymphoid follicles surrounded by marginal zone cells that infiltrate diffusely in lamina propria and into epithelium in small groups. |
| 5 | MALT lymphoma | Dense diffuse lamina propria infiltrate of marginal zone cells with prominent lymphoepithelial lesions. |

MALT, mucosa-associated lymphoid tissue.
after sufficient time has elapsed since treatment of gastric MALT lymphoma, the possibility of residual gastric MALT lymphoma cannot be ruled out, albeit normal histopathologic examination results [35].

**Follow-up of gastric MALT lymphoma**

Complete remission of gastric MALT lymphoma can be achieved at 80–86% by *H. pylori* eradication therapy [42,43]. *H. pylori* eradication therapy is also considered as a primary treatment option for *H. pylori*-negative gastric MALT lymphoma [44]. *H. pylori* eradication was confirmed at least 4 weeks after *H. pylori* eradication therapy, and follow-up test with EGD, histology, and EUS were performed every 3 months until complete remission was achieved. Complete remission after *H. pylori* eradication therapy is slow and may take more than a year in some cases [45]. The number of tumor cells is decreased after *H. pylori* eradication therapy; hence, a sufficient amount of biopsy specimens must be obtained. Complete remission can be determined if histologic remission is identified in two consecutive biopsy results.

After remission, lesions of gastric MALT lymphoma changed to atrophic and whitish discolored mucosa [46] (Fig. 3). Recovery of gastric pit and microvascular structures, which were destroyed and disappeared, can be seen on magnifying endoscopy [47]. The whitish discolored mucosa with definite vascular network showed no recurrence after remission, but the granular mucosa without definite vascular network more likely recurs after remission [48].

After remission, gastric MALT lymphoma can recur regardless of *H. pylori* reinfection, and it has a long-term recurrence rate of 7–8%, and the recurrence rate is 2.2% annually [9,42]. Because the risk of developing metachronous gastric adenocarcinoma is increased and this risk persists after several years in patients with gastric MALT lymphoma [49], annual follow-up EGD is recommended after remission of gastric MALT lymphoma. However, there is a lack of studies about how long follow-up EGD should be performed. Because the endoscopic findings of recurred...
gastric MALT lymphoma are non-specific and variable, sufficient biopsies at multiple sites must be performed.

**Conclusion**

The early diagnosis of gastric MALT lymphoma is difficult because its symptoms and endoscopic findings are nonspecific. Therefore, if an abnormal mucosa is observed during EGD, gastric MALT lymphoma should be considered, and multiple biopsies and EUS should be performed. In addition, the possibility of false-negative results of endoscopic biopsy should be considered, and more invasive tissue biopsy, such as EUS-FNA, EMR, and ESD, may be necessary. EUS can be used not only for diagnosis, but also for prediction of treatment response. Regular follow-up EGD and EUS are required after treatment of gastric MALT lymphoma.

**Acknowledgements**

This work was supported by a grant from Chunma Medical Research Foundation, Korea, 2017.

**Conflicts of interest**

No potential conflicts of interest relevant to this article was reported.

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Si Hyung Lee, https://orcid.org/0000-0001-7221-7506

**References**

Park BS and Lee SH  Endoscopic features in diagnosis and follow-up of gastric MALT lymphoma

Forefoot disorders are often seen in clinical practice. Forefoot deformity and pain can deteriorate gait function and decrease quality of life. This review presents common forefoot disorders and conservative treatment using an insole or orthosis. Metatarsalgia is a painful foot condition affecting the metatarsal (MT) region of the foot. A MT pad, MT bar, or forefoot cushion can be used to alleviate MT pain. Hallux valgus is a deformity characterized by medial deviation of the first MT and lateral deviation of the hallux. A toe spreader, valgus splint, and bunion shield are commonly applied to patients with hallux valgus. Hallux limitus and hallux rigidus refer to painful limitations of dorsiflexion of the first metatarsophalangeal joint. A kinetic wedge foot orthosis or rocker sole can help relieve symptoms from hallux limitus or rigidus. Hammer, claw, and mallet toes are sagittal plane deformities of the lesser toes. Toe sleeve or padding can be applied over high-pressure areas in the proximal or distal interphalangeal joints or under the MT heads. An MT off-loading insole can also be used to alleviate symptoms following lesser toe deformities. Morton’s neuroma is a benign neuroma of an intermetatarsal plantar nerve that leads to a painful condition affecting the MT area. The MT bar, the plantar pad, or a more cushioned insole would be useful. In addition, patients with any of the above various forefoot disorders should avoid tight-fitting or high-heeled shoes. Applying an insole or orthosis and wearing proper shoes can be beneficial for managing forefoot disorders.

Keywords: Conservative treatment; Forefoot disorder; Insole; Orthosis; Shoes

Introduction

Forefoot disorders are extremely prevalent in the general population and can hinder patients’ activities of daily living. In cases where severe pain exists during gait, patients are reluctant to walk around, which can lead to poor physical function [1,2]. In many cases, forefoot disorders are caused by wearing ill-fitting or high-heeled shoes, altered foot alignment, and foot arthropathy [3,4]. Although there are several forefoot disorders, metatarsalgia, hallux valgus, hallux limitus/rigidus, lesser toe deformities (hammer, claw, and mallet toes), and Morton’s (interdigital) neuroma are common. For the management of these disorders, conservative treatment is usually attempted prior to surgical intervention. Conservative treatments include corrective shoes, the application of insoles or orthoses such as pads or supports, oral medications, and steroid injections [5]. Despite the high incidence of forefoot disorders, their conservative treatments have rarely been studied. Thus, in this review, we aim to summarize common forefoot disorders and present conservative treatments by focusing on shoe modifications and the application of insoles or orthoses (Table 1).

Metatarsalgia

Metatarsalgia refers to pain in the plantar aspect of the foot in the
region of the metatarsal (MT) head, particularly in the second, third, and fourth rays [6]. During the toe-off phase of gait, most of the pressure is concentrated in this area. A deteriorated biomechanical condition of the foot can increase pressure on the MT head during weight bearing, resulting in metatarsalgia. Metatarsalgia can be divided into three types: primary, secondary, and iatrogenic [6]. The incidence of metatarsalgia is approximately 5–36% [7].

Primary metatarsalgia is caused by anatomical abnormalities of the MT and the relationships between the MT and other parts of the foot. The common causes of primary metatarsalgia include first ray insufficiency [8], a long second MT [9], and increased MT declination caused by pes cavus [8]. First ray insufficiency is caused by inability of the first ray to accept loads during weight bearing, leading to transfer pressure to the lesser MT [8]. First ray insufficiency occurs due to several conditions, such as hallux valgus, pes planus, and hypermobility of the first metatarsophalangeal (MTP) joint [10]. The long second MT shifts loads during weight bearing from the first to the second ray [9]. Moreover, in pes cavus, MT declination increases and pressure with heel strike focuses on the MT head and heel without adequate lateral plantar midfoot support [8]. These conditions increase lesser MT load and cause metatarsalgia.

Secondary metatarsalgia is induced by indirect overloading of the forefoot [6]. Foot trauma can alter foot alignment, causing angular or rotational MT displacement. Fracture or injury to the supporting structures of the MTP joint (plantar plate and collateral ligaments) deteriorates the foot’s biomechanical alignment, leading to forefoot instability and pain [6]. Other conditions, including hallux rigidus, Morton’s neuroma, tarsal tunnel syndrome, Freiberg infarction, and chronic inflammatory diseases can increase forefoot load without direct load on the MT [8,11-13].

Iatrogenic metatarsalgia arises as a sequela of prior forefoot surgery, such as altered MT position after proximal MT osteotomy and excessive MT shortening after MT osteotomy or hallux valgus surgery (Fig. 1) [14-16]. Complications after forefoot surgery, including nonunion, malunion, or avascular necrosis, can cause iatrogenic metatarsalgia [14-16].

To effectively treat metatarsalgia, clinicians should diagnose its

<p>| Table 1. Summary of conservative treatment for forefoot disorders (other than shoe modification) |</p>
<table>
<thead>
<tr>
<th>Forefoot disorder</th>
<th>Conservative treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metatarsalgia</td>
<td>Metatarsal pad, metatarsal bar, and forefoot cushion</td>
</tr>
<tr>
<td>Hallux valgus</td>
<td>Toe spreaders</td>
</tr>
<tr>
<td>Hallux limitus/rigidus</td>
<td>Kinetic wedge</td>
</tr>
<tr>
<td>Hammer, claw, and mallet toes</td>
<td>Toe sleeve or padding, metatarsal off-loading insole</td>
</tr>
<tr>
<td>Morton’s (interdigital) neuroma</td>
<td>Metatarsal bar, planar pad, and more cushioned thicker insole</td>
</tr>
</tbody>
</table>

Fig. 1. (A) Metatarsal shortening of first metatarsal on right foot due to hallux valgus recurrence after hallux valgus surgery. (B) Callosity under second and third metatarsal head due to altered metatarsal position after hallux valgus surgery.
causal factor and focus on solving it. For symptomatic relief, an MT pad made of rubber, polyurethane, or silicone can be applied. The pad reduces pressure under painful MT heads by spreading it to a larger area, improving functional ability [17-19]. In 2005, Hsi et al. [18] reported that the optimal method is to apply an MT pad just proximal to the MT head. It also elevates the horizontal arch of the forefoot, which can widen the space between MT heads, reducing interdigital nerve compression and irritation [18]. Use of an MT bar or forefoot cushion is also effective for controlling metatarsalgia [20].

Hallux valgus

Hallux valgus, the most common foot deformity, is characterized by medial deviation of the first MT and lateral deviation of the hallux. The incidence of hallux valgus is approximately 23% in 18–65-year-olds and 35% in people over 65 years of age [21]. When the hallux valgus angle, defined as the angle between the shaft axis of the first MT and the proximal phalanx of the hallux, is greater than 15°, patients are diagnosed with hallux valgus. Hallux valgus can be categorized as mild (15–20°), moderate (21–39°), and severe (≤40°) [22]. In severe cases, subluxation of the first MTP joint can be involved. Pain in the MTP joint, especially during weight bearing, is routinely seen in patients with hallux valgus [22].

The first ray bears a significant amount of weight because it maintains the position of the medial arch [23]. Several factors that deteriorate the integrity of the first ray, such as restrictive footwear, foot deformities, and pes planus, can be ascribed to the occurrence of hallux valgus [24]. The much higher prevalence of hallux valgus in women (women:men=15:1) is associated with the differential use of tight-fitting and high-heeled shoes [25]. Moreover, the fact that women have higher rates of ligamentous laxity, which disrupts the integrity of the first ray, seems to contribute to the different incidence in men and women [26].

Prior to surgical correction, conservative treatment should be initiated. Patients should avoid tight-fitting or high-heeled shoes and wear soft and wide-toed shoes instead [22]. A toe spreader, valgus splint, and bunion shield are suggested treatments for hallux valgus (Fig. 2) [22]. The toe spreader separates the first toe from the second toe and reduces pain caused by the bunion, reducing protrusion of the MT head [27]. Many types of ready-made toe spreaders are being applied in patients with hallux valgus. In 2008, Tehraninasr et al. [27] recruited 30 patients with hallux valgus and compared the effects of a toe separator made of plastazote material with a semi-rigid total contact insole and those of a valgus splint. After 3 months, pain intensity was significantly reduced in 15 patients who were managed with the toe separator, whereas the change in pain was not significant in the other 15 patients treated with the valgus splint. On the other hand, the hallux valgus angle was not significantly reduced in either group. In addition, ready-made toe spreaders do not accurately fit the toe spaces of each patient; thus, the toe axis cannot be accurately corrected. In 2018, Cha et al. [28] designed personalized toe spreaders with three-dimensional scanning and printing and successfully applied them in eight patients with hallux valgus.

Hallux limitus/rigidus

Hallux limitus refers to restricted sagittal range of motion (ROM) of the first MTP joint. Also, hallux rigidus is defined as a condition in which sagittal ROM is completely absent [29-31]. Hallux limitus/rigidus occurs in 35–60% of peoples over 65 years old [32-34]. In addition, a functional reduction in ROM of the first MTP joint that occurs during walking with no structural limitations is referred to as functional hallux limitus [34].
The predisposing factors of hallux limitus/rigidus include, osteoarthritis, trauma, rheumatoid arthritis, age, female sex, hypermobile first ray, hallux valgus, and pes planus [33,35-37]. The first MTP joint bears about 60% of one’s body weight [38]. Pes planus leads to excess weight load on the first MTP joint, which causes arthritic changes in the first MTP joint.

A kinetic wedge foot orthosis can be applied to treat hallux limitus or rigidus [39,40]. This involves the use of a cut out under the first MT head, which seems to increase dorsiflexion at the first MTP joint and allow the first ray to plantarflex more freely. The kinetic wedge foot orthosis reduces plantar pressure under the first MTP joint. In 2003, Rambarran et al. [40] reported that plantar pressure under the first MTP joint during gait was significantly reduced after the application of a kinetic wedge foot orthosis. The addition of a rocker sole also helps relieve symptoms [41].

Hammer, claw, and mallet toes

Hammer, claw, and mallet toes are sagittal plane deformities of the lesser toes. The incidence of these deformities is approximately 30% [42]. Hammer toe refers to a flexion deformity at the proximal interphalangeal (PIP) joint of the toe accompanied by a slight MTP joint extension deformity (Fig. 3) [5]. Claw toe is defined as a hyperextension deformity at the MTP joint and secondarily having flexion deformity in the PIP and distal interphalangeal (DIP) joints (Fig. 4) [5]. Mallet toe is defined as a flexion deformity at the DIP joint. Difficult in wearing shoes (impingement of the toes on the shoe box) and metatarsalgia can occur during these deformities (Fig. 5) [5]. These deformities of the lesser toe are associated with ill-fitting or high-heeled shoes [43]. Hallux valgus can also contribute to the formation of lesser toe deformities [44]. Shortening of the first ray by hallux valgus slackens the plantar fascia and weakens the windlass effect on the first toe, which leads to greater strain on the lesser toes [44]. This makes the supporting structures of the lesser toe more likely to fail. Pathologies such as diabetes mellitus, neuromuscular disorders, and inflammatory arthritis can also cause lesser toe deformities [43]. Clinicians should recommend that patients wear wider shoes with a larger toe box [45]. A toe sleeve or padding can be applied over high-pressure areas of the PIP or DIP joints or under the MT heads [46]. MT off-loading insoles can also be used to alleviate symptoms following lesser toe deformities [47]. However, there is paucity of clinical data on the effect of conservative management of lesser toe deformities; thus, a clinical trial should be performed on this theme in the future.
Morton’s (interdigital) neuroma

Morton’s neuroma is a compression neuropathy of the plantar digital nerve with associated perineural fibrosis [48]. Morton’s neuroma reportedly affects 30% of the population and is predominant in the female sex (female: male = 4:1) [49]. It most commonly affects the third space (66% of cases), followed by the second (32%) and fourth spaces (2%) [50]. Its symptoms are a burning and tingling sensation in the forefoot, with occasional numbness in the affected toe [48]. Morton’s neuroma, a thickening of the interdigital nerve, occurs just distal to the MT transverse ligament and before bifurcation of the digital nerves. Entrapment of the interdigital nerve between the intermetatarsal ligaments is considered the key factor in the occurrence of Morton’s neuroma [51]. It is related to various overload mechanisms and the use of inadequate footwear [52]. Morton’s neuroma can be diagnosed by a physical examination. The application of plantar pressure on the area between and just proximal to the MT heads should replicate the patient’s usual pain [48]. Moreover, concomitant lateral, dorsal, and plantar compression of the MT heads can produce an audible and painful Mulder click [48].

For treatment, patients should favor shoes with a lower heel and wider toe box [49]. An MT bar on the sole of the shoe reduces pressure loading on the space between MT heads and transfers it more proximally [53]. Plantar pads placed to the insole proximal to the neuroma elevate the MT head and decrease interspace pressure [53]. The use of a more cushioned thicker insole can decrease the pressure and impact on the intermetatarsal space [53].

Conclusion

In clinical practice, many patients complain of forefoot pain or deformity. The modification of shoes and application of insoles or orthoses can be beneficial for managing forefoot disorder. However, clinical data or evidence of these conservative treatment methods are insufficient. To clarify the utility of these treatments in forefoot disorders, further studies are needed. This review will help clinicians consider various tools for treating forefoot disorders.

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

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Efficacy of ramosetron in combination with polyethylene glycol of preparing for a colonoscopy

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Department of Internal Medicine, Y eungnam University College of Medicine, Daegu, Korea

Background: Because of its efficacy and safety, polyethylene glycol (PEG) is generally used to prepare for colonoscopy. However, the side effects of PEG, including nausea, vomiting, abdominal discomfort, pain, and general weakness, tend to decrease patient compliance and satisfaction. The aim of this study is to investigate the efficacy and safety of PEG with 0.1 mg ramosetron on colonoscopy patients who had difficulty taking PEG due to side effects or large volume.

Methods: From January to August in 2012, 28 patients who visited Yeungnam University hospital for a colonoscopy were prospectively enrolled. All enrolled patients were previous history under-went colonoscopy using PEG only in our hospital. The efficacy and safety of ramosetron were assessed through the use of a questionnaire, and compared previous bowel preparation.

Results: Compared to previous examination, the patients using the ramosetron reported less nausea, vomiting, abdominal discomfort, and abdominal pain, as well as a higher degree of compliance and satisfaction of the patient. There were no side effects reported with the use of ramosetron. However, overall bowel preparation quality was not better than the previous examination.

Conclusion: In case of the use of ramosetron in combination with PEG for bowel preparation, patients experienced a higher rate of compliance and tolerance. Looking forward, ramosetron may become an option of pretreatment for bowel preparation.

Keywords: Bowel preparation; Colonoscopy; Polyethylene glycol; Ramosetron

Introduction

According to the increase of the proportions of colon disease year after year, the importance of colonoscopy examinations is growing. Besides, the general use of colonoscopies has been suggested for colon cancer screening. Thorough bowel preparation is necessary for the safe and accurate completion of the colonoscopy examination. Actually, diagnostic accuracy and therapeutic safety of the colonoscopy relies on the quality of the bowel preparation [1-5].

Though appropriate preparation is generally defined as one that allows the detection of colonic polyps 5 mm or larger [6], this definition does not consider the shape of the lesions, and it is well known that flat lesions are more difficult to detect. The cecal intubation rate and adenoma detection rate are two of the chief quality colonoscopic indices. Those have close relation with the quality of bowel preparation [7].

Inappropriate bowel cleaning can cause low detection rates of incipient and advanced adenomas, flat lesions, and flat adenomas [7-10]; it can also cause a higher rate of canceled procedures with increased costs, prolonged procedures, and a higher risk of complications [11].

Bowel preparation is one of the concerns that negatively affect the willingness of patients to receive the colonoscopy procedure.
Compliance to the preparation process is a main important factor for success colonoscopy, as compliance affects the quality of the bowel preparation. But, the preparation process has limitations due to side effects, and the low tolerance for the taste and the large amount of solution, which are the leading causes for avoiding the complete bowel preparation [14]. Polyethylene glycol (PEG) solution has been the favored bowel preparation agent to use prior to performing diagnostic and therapeutic colonoscopy procedures on the colon and rectum.

Ramosetron is a selective serotonergic 5-hydroxy-tryptamine (HT) receptor-3 antagonist that is used to prevent and treat postoperative or chemotherapy-induced nausea and vomiting [15,16]. The author supposes that ramosetron can improve a patient’s tolerance and satisfaction regarding the PEG solution. However, the efficacy and tolerability of PEG with or without ramosetron has not been investigated. Based on that, we conducted a prospective, pilot study to evaluate the tolerability, and efficacy of ramosetron as an adjuvant in the PEG solution for colonoscopy preparation who had difficulty taking PEG due to side effects and large volume.

Materials and methods

1. Patients
Twenty-eight outpatients aged 17 to 80 years, scheduled for elective colonoscopy at Yeungnam University hospital from January to August 2012, were enrolled. All enrolled patients had experience with the bowel preparation process that is required for a colonoscopic examination in our hospital. The patients had negative willingness on previous colonoscopy due to difficulty drinking the PEG solution, regardless of degree of bowel preparation and PEG dosage. Patients were excluded if they had a megacolon, bowel obstruction, ileus, or other severe comorbidities that might prevent the colonoscopy from occurring. Patients were also excluded if they had colonoscopy for emergency purposes. The study was approved by the Institutional Review Committee of Yeungnam University hospital (YUH-12-015-M10).

2. Methods
PEG administration was started 12 hours before the colonoscopy examination. All patients were instructed to drink 4 liters of PEG solution if they had no side effects or difficulties. After starting to drink the PEG solution, patients were only allowed to have water. Ramosetron of 0.1 mg (Nasea®; Astellas, Tokyo, Japan) was administered one time, and it was given 1 hour before drinking the PEG solution.

Before receiving the current colonoscopy examination, patients filled out a questionnaire to estimate their acceptability and tolerability regarding the bowel preparation. In regards to tolerance, patients were evaluated using each quartile of the total amount PEG solution they drank. Patients were also questioned as to whether they experienced any side effects associated with the current bowel preparation such as nausea, vomiting, abdominal discomfort & pain. They were also questioned about their satisfaction with the current bowel preparation. In the same enrolled patients, previous total amount PEG solution, side effects were also investigated in a written questionnaire before receiving the current colonoscopy. In other words, we divided the previous PEG only group and current PEG+ramosetron group in the same subject.

To estimate the efficacy of the bowel preparation, patients were evaluated using bowel preparation quality on the colonoscopic examination by experienced colonoscopist. Bowl preparation status was categorized into four groups as clear, fluid that can be sucked away, small amount of feces, and large amount of feces. Optimal bowel preparation status defined as the two groups including clear and fluid that can be sucked away. After the colonoscopy procedure, the colonoscopist filled out a questionnaire about the current bowel preparation status, compared the results of previous bowel preparation status, and calculated cecal intubation rate, withdrawal time, adenoma detection rate, and optimal bowel preparation status rate after reviewing colonoscopy images.

3. Statistical analysis
The primary endpoints of this study were the patients’ tolerance and efficacy of the PEG-solution. Continuous variables were reported as mean±standard deviations and analyzed using independent t-tests. Results are expressed as counts and percentages as categorical data, and analyzed using Pearson’s Chi-square test or Fisher’s exact test, as appropriate. A p-value <0.05 was considered statistically significant.

Results
The baseline characteristics of current PEG+ramosetron group are presented in Table 1. The mean age of enrolled patients was 48.79±14.97 years old. Male to female ratio was 1:1.54. Of the 28 patients, 11 patients had underlying diseases such as 5 diabetes mellitus (17.9%), 6 hypertension (21.4%), 5 cancer (17.9%), 2 asthma (7.1%), and 3 heart failure (10.7%). Of the total patients, 13 patients (46.4%) had a previous history of abdominal surgery. The mean previous colonoscopy interval time was 26.3±17.7 months. The mean cecal intubation time and withdrawal time

https://doi.org/10.12701/yujm.2019.00080
were 4.1±2.1 and 6.7±2.4 min, respectively.

Concerning the PEG volume, 12 of the 28 patients (42.9%) were able to finish 100% (4 L) of the PEG solution in the previous PEG only group. However, 22 of the 28 patients (78.6%) were able to finish 100% of the PEG solution in the PEG+ramosetron group (p=0.019). The number of patients who finished less than half the amount of the PEG solution are 5 (17.8%) and 1 (3.5%) in previous PEG only group and PEG+ramosetron group, respectively. The patients' acceptability in these two groups was significantly different (Table 2).

With regard to side effects of the bowel preparation, several symptoms were assessed in the questionnaire, including nausea, vomiting, abdominal discomfort and abdominal pain. Side effects were more frequently found in the previous PEG only group than in the PEG+ramosetron group (p<0.001). Of the total 28 patients, 18 (64.3%) patients did not complain of any side effects in PEG+ramosetron group (Table 2).

The authors investigated the satisfaction of patients with the bowel preparation method. The proportion of excellent satisfaction in the PEG+ramosetron group (64.3%) was higher than that in the previous PEG only group (64.3% vs. 19.7%, p<0.001, respectively) (Table 2).

### Table 1. Baseline characteristics of the PEG+ramosetron group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PEG+ramosetron group (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>48.8±14.9</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11 (39.2)</td>
</tr>
<tr>
<td>Female</td>
<td>17 (60.7)</td>
</tr>
<tr>
<td>Presence of comorbidities</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>5 (17.9)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>6 (21.4)</td>
</tr>
<tr>
<td>Cancer</td>
<td>5 (17.9)</td>
</tr>
<tr>
<td>Asthma</td>
<td>2 (7.1)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>3 (10.7)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.68±8.74</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59.49±11.24</td>
</tr>
<tr>
<td>BMI</td>
<td>21.77±3.44</td>
</tr>
<tr>
<td>History of abdominal operation</td>
<td>13 (46.4)</td>
</tr>
<tr>
<td>Previous colonoscopy interval time (mon)</td>
<td>26.3±17.7</td>
</tr>
<tr>
<td>Cecal intubation time (min)</td>
<td>4.1±2.1</td>
</tr>
<tr>
<td>Withdrawal time (min)</td>
<td>6.7±2.4</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation or number (%), unless otherwise specified. PEG, polyethylene glycol; BMI, body mass index.

### Table 2. Comparison of the differences between previous PEG only group and PEG+ramosetron group at the same patients

<table>
<thead>
<tr>
<th></th>
<th>Previous PEG only (n = 28)</th>
<th>PEG+ramosetron (n = 28)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG taking dose (%)</td>
<td></td>
<td></td>
<td>0.019</td>
</tr>
<tr>
<td>100</td>
<td>12 (42.9)</td>
<td>22 (78.6)</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>11 (39.3)</td>
<td>5 (17.9)</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>5 (17.8)</td>
<td>1 (3.5)</td>
<td></td>
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<tr>
<td>Side effect</td>
<td></td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>27 (96.4)</td>
<td>8 (28.6)</td>
<td></td>
</tr>
<tr>
<td>Abdominal discomfort/pain</td>
<td>1 (3.6)</td>
<td>2 (7.2)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0 (0.0)</td>
<td>18 (64.3)</td>
<td></td>
</tr>
<tr>
<td>Patient satisfaction</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Excellent</td>
<td>3 (10.7)</td>
<td>18 (64.3)</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>17 (60.7)</td>
<td>7 (25)</td>
<td></td>
</tr>
<tr>
<td>Unsatisfactory</td>
<td>8 (28.6)</td>
<td>3 (10.7)</td>
<td></td>
</tr>
<tr>
<td>Bowel preparation</td>
<td></td>
<td></td>
<td>0.175</td>
</tr>
<tr>
<td>Clear</td>
<td>8 (28.6)</td>
<td>16 (57.2)</td>
<td></td>
</tr>
<tr>
<td>Fluid that can be sucked away</td>
<td>14 (50.0)</td>
<td>9 (32.1)</td>
<td></td>
</tr>
<tr>
<td>Small amounts of feces</td>
<td>4 (14.3)</td>
<td>2 (7.1)</td>
<td></td>
</tr>
<tr>
<td>Large amounts of feces</td>
<td>2 (7.1)</td>
<td>1 (3.6)</td>
<td></td>
</tr>
<tr>
<td>Optimal bowel preparation</td>
<td>22 (78.6)</td>
<td>25 (89.3)</td>
<td>0.052</td>
</tr>
<tr>
<td>Quality of colonoscopy</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cecal intubation rate</td>
<td>27 (96.4)</td>
<td>27 (96.4)</td>
<td>p&gt;0.999</td>
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<tr>
<td>Adenoma detection rate</td>
<td>8 (28.6)</td>
<td>7 (25.0)</td>
<td>0.118</td>
</tr>
</tbody>
</table>

Values are presented as number (%).

PEG, polyethylene glycol.
The efficacy of the bowel preparation was categorized into four groups as clear, fluid that can be sucked away, small amount of feces and large amount of feces. As much as 78.6% of bowel preparation in the previous PEG only group was optimal and 89.3% of bowel preparation in PEG+ramosetron group was optimal ($p=0.052$). Although not statistically significant, both groups showed a comparable overall assessment of bowel preparation ($p=0.175$) (Table 2).

The cecal intubation rate and adenoma detection rate, which are quality indexes of colonoscopy quality, did not differ between the two groups. The cecal intubation rate accounted for 96.4% of both two groups, except for one patient with stenosis with Crohn's disease. Adenoma detection rate were 28.6% in previous PEG only group and 25.0% in the PEG + ramosetron group, which are not statistical significantly ($p=0.118$).

**Discussion**

Bowel preparation before colonoscopy is usually considered as uncomfortable by patients. It is common for patients to complain that the bowel preparation is harder than the actual colonoscopy procedure. Accordingly, many kinds of agents have been studied in order to achieve comfortable and effective bowel preparation. Many reports about PEG solution have concluded that PEG is more effective and better tolerated than the combination of diet and laxative regimens, high-volume balanced electrolyte solutions, and mannitol-based solutions [17-20]. Even if PEG is usually well-tolerated, 5-15% patients who take PEG do not complete the preparation because of the bad taste, large volume or side effects [21]. In particular, patients experiencing difficulty with PEG administration and side effects may have a negative willingness of colonoscopy, leading to poor compliance and avoidance of examination.

There are several studies that have evaluated the effects of prokinetics on bowel preparation. Abdullah et al. reported that the adjunct use of clebopride in PEG solution for bowel preparation tended to increase the acceptability, tolerability and efficacy [22]. Tajika et al. revealed that mosapride citrate can be an effective and safe adjunct to PEG solution which brings about improved bowel preparation status [23]. However, it has not been fully evaluated to add-on prokinetic agents with PEG in terms of the efficacy and safety of colonic cleansing. In addition, as the difficulty and side effects during PEG administration may increase the avoidance of colonoscopy, safer and more effective adjunctive agents are required.

Ramosetron (Nasea®, Astellas), a new generation 5-HT3 antagonists developed newly in Japan, has a higher potency and longer lasting antiemetic effect than first-generation 5-HT3 antagonists [24,25]. Three-dimensional molecular modeling studies have revealed that ramosetron occupies the 5-HT binding site of 5-HT3 receptors with long lasting antagonism [26]. Ramosetron is commonly used to treat postoperative nausea and vomiting or chemotherapy-induced nausea and vomiting [27].

The present study showed that patient acceptability was superior in the PEG+ramosetron group compared to the previous PEG only group ($p=0.019$). With regard to side effects, ramosetron reduced the presence of nausea and vomiting ($p<0.001$). In regard to patient satisfaction, the proportion of excellent satisfaction in the PEG+ramosetron group was higher than that in the previous PEG only group ($p<0.001$). As to the efficacy of bowel preparation assessed by the colonoscopist, both groups showed comparable results ($p=0.175$). This was consistent with results of other reports that showed no statistically significant differences between the regimens for bowel preparation [28-32].

Although good bowel preparations and PEG doses are known to be interrelated, no difference in bowel preparation status revealed between two groups. The reasons for this are as follows. First, the PEG dosage is measured in quarters, making it difficult to accurately dose PEG. That is, the difference in the amount of 1L of PEG in each quartile may have affected the different in the degree of bowel preparation. Second, differences in the dosing interval may have affected the bowel preparation. Non-compliance of dosing interval was not checked. Third, the previous PEG dose is likely to be inaccurate due to a colonoscopy interval of 2 years or more.

Generally, the PEG solution is very effective for bowel preparation before colonoscopy. But patients show a somewhat low tolerance due to the large amount of solution and the side effects that include nausea, vomiting and abdominal pain. In the present study, ramosetron could increase the tolerance and decrease various side effects. In addition, ramosetron may help to reduce negative willingness about colonoscopy by increasing the satisfaction of taking PEG.

There are some limitations in this study. First, although it is a prospective study, it is a small population-pilot study without a definite control group. No appropriate control group to compare with PEG+ramosetron group only was established. Second, recall bias can occur. As I aforementioned, depending on the memory, the data was collected in a form that filled the questionnaire with total amount of PEG solution and the side effects that occurred during the previous colonoscopy. Third, the effect of ramosetron can be overestimated. Fourth, due to the chart review format, accurate assessment of previous patients' characteristics and quality of colonoscopy including cecal intubation time and withdrawal.
time may be insufficient. Finally, grading of side effects is not evaluated, although no severe adverse effects were found to stop colonoscopy. However, this study was the first to compare efficacy with previous PEG alone group using PEG+ramosetron for bowel preparation in patients with difficult taking PEG.

In conclusion, similar to several prokinetics that were reported at previous study, ramosetron can be a good adjunctive agent for bowel preparation when used with PEG solution.

Conflicts of interest

No potential conflicts of interest relevant to this article was reported.

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Impact of calcineurin inhibitors on rat glioma cells viability

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Background: Although kidney transplantation outcomes have improved dramatically after using calcineurin inhibitors (CNIs), CNI toxicity continues to be reported and the mechanism remains uncertain. Here, we investigated the neurotoxicity of CNIs by focusing on the viability of glioma cells.

Methods: Glioma cells were treated with several concentrations of CNIs for 24 hours at 37°C and their cell viability was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

Results: Exposure to 0, 0.25, 0.5, 2.5, 5.0, and 10.0 mM concentrations respectively showed 100%, 64.3%, 61.3%, 68.1%, 62.4%, and 68.6% cell viability for cyclosporine and 100%, 38.6%, 40.8%, 43.7%, 37.8%, and 43.0% for tacrolimus. The direct toxic effect of tacrolimus on glioma cell viability was stronger than that of cyclosporine at the same concentration.

Conclusion: CNIs can cause neurological side effects by directly exerting cytotoxic effects on brain cells. Therefore, we should carefully monitor the neurologic symptoms and level of CNIs in kidney transplant patients.

Keywords: Calcineurin inhibitors; Glioma cell; Kidney transplantation; Neurotoxicity

Introduction

The results of kidney transplantation (KT) have improved dramatically after the use of calcineurin inhibitors (CNIs) [1]. However, some complications of CNIs have been also reported [2]. Nephrotoxicity was a majorly reported side effect of CNIs that were assessed through laboratory or allograft biopsy findings [3,4]; however, it could be mitigated by controlling CNI trough levels. Unfortunately, mild neurologic symptoms of CNI neurotoxicity, such as tremors, agitation, insomnia, anxiety, and paresthesia, could easily go unnoticed [5,6]. In such cases, mild symptoms could worsen to cortical blindness, seizures, and encephalopathy, which could cause lethal damage to the brain. Recently, CNI neurotoxicity was diagnosed in computed tomography and magnetic resonance imaging studies, which showed morphological findings such as hypodensity of the white matter, cerebral edema, metabolic encephalopathy, and hypoxic damages [7-9]. Paradoxically, cyclosporine has been found to protect the brain from ischemia–reperfusion injury in animal models; however, the mechanism of CNI neurotoxicity is not yet fully understood.

Here, we investigated the neurotoxicity of two CNIs, cyclosporine, and tacrolimus, on the viability of glioma cells.

Materials and methods

1. Cell culture
Rat glioma cells (Korean Cell Line Bank, Seoul, Korea) were
cultured in Dulbecco’s modified Eagle’s medium (WelGENE, Daegu, Korea) supplemented with 10% fetal bovine serum (WelGENE) and 1% penicillin–streptomycin (HyClone, Logan, UT, USA). Cells were grown in 10-cm diameter culture plates at 37°C under humidified conditions containing 5% CO₂/95% air.

2. Measuring the viability of glioma cells
Cytotoxicity was estimated by the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay, which is based on the cleavage of a tetrazolium salt by mitochondrial dehydrogenases in viable cells. Glioma cells were plated in a 96-well plate at an initial density of 104 cells/well. After 24 hours, the medium was replaced with fresh medium containing various concentrations (0, 0.25, 0.5, 2.5, 5.0, and 10.0 mM) of cyclosporine (Novartis Pharma AG, Basel, Switzerland) or tacrolimus (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan). The drugs were dissolved in dimethyl sulfoxide, and in the experiment, the cells were diluted in DMEM medium and treated with the required concentrations. Cells were incubated at 37°C for 24 hours. During the last 4 hours, the cells were incubated with 20 μL of MTT stock solution (5 mg/mL). The plates were shaken for 10 to 15 minutes in the dark. The optical density was measured at 570 nm using the microplate reader 550 (Bio-Rad, Lab., Hercules, CA, USA), and the relative cell viability was expressed using the following equation:

\[
\text{Cell viability (\%)} = \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \times 100
\]

3. Statistical analysis
The values are expressed as mean±standard deviation. Statistical evaluation of the significant difference between the means was performed using Student’s t-test. SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. \(p<0.05\) was considered to be significant.

Results
Substantial morphological changes were observed in glioma cells when they were treated with cyclosporine or tacrolimus.

The cells were observed at a magnification of ×400 using the DMi1 inverted microscope (Leica Microsystems, Wetzlar, Germany). Cells were detached and floated to the top of the culture dish; however, a monolayer was not formed (Fig. 1).

For cyclosporine, cell viabilities were as follows: 100±0.1% at 0 mM/L cyclosporine control, 64.3±18.5% \((p<0.05\) vs. control) at 0.25 mM/L cyclosporine, 61.3±12.0% \((p<0.01\) vs. control) at 0.50 mM/L cyclosporine, 68.1±18.8% \((p<0.05\) vs. control) at 2.5 mM/L cyclosporine, 62.4±24.5% \((p<0.05\) vs. control) at...
5.0 mM/L cyclosporine, and 68.6±19.5% (p<0.05 vs. control) at 10.0 mM/L cyclosporine.

For tacrolimus, cell viabilities were as follows: 100±0.1% at 0 mM/L tacrolimus control, 38.6±29.4% (p<0.05 vs. control) at 0.25 mM/L tacrolimus, 40.8±26.5% (p<0.05 vs. control) at 0.50 mM/L tacrolimus, 43.7±21.7% (p<0.05 vs. control) at 2.5 mM/L tacrolimus, 37.8±27.7% (p<0.01 vs. control) at 5.0 mM/L tacrolimus, and 43.0±29.8% (p<0.05 vs. control) at 10.0 mM/L tacrolimus.

Our study showed that the direct toxic effect of tacrolimus was stronger on brain cells than that of cyclosporine at the same concentration; however, no significant difference was observed between the two groups.

**Discussion**

Although advances in immunosuppressants have resulted in remarkable improvements with respect to allograft acceptance and patient survival rates in KT [1], the use of CNIs, such as cyclosporine and tacrolimus, has caused several side effects including nephrotoxicity [4], post-transplant bone disease [10], hepatotoxicity [11], hypertension [12], diabetes [13], dyslipidemia [2], and neurological side effects [14,15].

The clinical characteristics and mechanisms of CNI neurotoxicity are still controversial and poorly understood. Recently, hypomagnesemia [16], hypocholesterolemia [17], severe vasoconstriction [17], and hypertension via the inhibition of nitric oxide production were reported as precipitating factors [18]; however, they do not sufficiently explain the mechanism of CNI neurotoxicity. In our previous study, cyclosporine and tacrolimus were shown to exhibit cytotoxic effects on renal cells and osteoblasts [10,19,20]. Thus, cyclosporine and tacrolimus may also exhibit cytotoxic effects on brain cells. Here, we studied the direct cytotoxicity of cyclosporine and tacrolimus on glioma cell viability to understand the underlying mechanisms of neurotoxicity.

Our results demonstrated that treatment with cyclosporine and tacrolimus resulted in substantial morphological changes as well as cell death in glioma cells.

Although neurotoxicity is known to be frequently associated with CNI trough levels exceeding recommended levels, it also occurs during long-term treatment even when CNI concentrations are within the therapeutic target range. Toxicity may be also be related to the type and dose of CNIs. For instance, tacrolimus is well known to be more potent than cyclosporine. In our study, the direct toxic effect of tacrolimus on glioma cell viability was greater than that of cyclosporine at the same concentration; however, no significant difference was observed. The clinical predisposition and mechanisms of cyclosporine-induced neurotoxicity remain controversial and poorly understood. Further studies are needed to investigate the precipitating factors for CNI-induced central nervous system abnormalities in addition to elucidating the positive effects of CNIs.

Interestingly, cyclosporine was previously reported to improve cerebral ischemia-reperfusion injury in vivo, and low concentrations of cyclosporine were shown to be neuroprotective [21]. Contrastingly, high concentrations of cyclosporine can cause mitochondrial dysfunction, which can lead to the deterioration of energy production, increased oxidative stress [22], and rapid apoptotic or necrotic cell death. Therefore, properly controlling CNI concentrations will be important in preventing CNI neurotoxicity.

CNIs can cause neurological side effects via direct cytotoxic effects in rat glioma cells. Therefore, we should carefully monitor neurologic symptoms and levels of CNIs.

**Conflicts of interest**

No potential conflicts of interest relevant to this article was reported.

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**References**


Digital subtraction angiography vs. real-time fluoroscopy for detection of intravascular injection during transforaminal epidural block

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Background: Transforaminal epidural block (TFEB) is an effective treatment option for radicular pain. To reduce complications from intravascular injection during TFEB, use of imaging modalities such as real-time fluoroscopy (RTF) or digital subtraction angiography (DSA) has been recommended. In this study, we investigated whether DSA improved the detection of intravascular injection during TFEB at the whole spine level compared to RTF.

Methods: We prospectively examined 316 patients who underwent TFEB. After confirmation of final needle position using biplanar fluoroscopy, 2 mL of nonionic contrast medium was injected at a rate of 0.5 mL/s under RTF; 30 s later, 2 mL of nonionic contrast medium was injected at a rate of 0.5 mL/s under DSA.

Results: Thirty-six intravascular injections were detected for an overall rate of 11.4% using RTF, with 45 detected for a rate of 14.2% using DSA. The detection rate using DSA was statistically different from that using RTF (p=0.004). DSA detected a significantly higher proportion of intravascular injections at the cervical level than at the thoracic (p=0.009) and lumbar (p=0.011) levels.

Conclusion: During TFEB at the whole spine level, DSA was better than RTF for the detection of intravascular injection. Special attention is advised for cervical TFEB, because of a significantly higher intravascular injection rate at this level than at other levels.

Keywords: Analgesia; Complications; Epidural; Radiculopathy; Spine

Introduction

Transoraminal epidural block (TFEB) is an effective diagnostic and treatment option for spinal radicular pain [1]. The transoraminal approach is target-specific, compared with other approaches for epidural blocks [2]. Potential risks associated with TFEB include infection [3,4], dural puncture [5,6], bleeding, and intravascular injection [5]. To reduce complications resulting from intravascular injection of drugs, several methods have been proposed, including use of short-beveled or blunt-type needles, large-diameter needles, non-particulate steroids, or imaging modalities such as real-time fluoroscopy (RTF) or digital subtraction angiography (DSA) [7]. There are no case reports or studies about fatal neurologic events resulting from intravascular injection of non-particulate steroids, but administration of local anesthetics may cause rare complications in the central nervous system during a cervical TFEB. Local anesthetics depress respiration and consciousness during a cervical root block [8].

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https://doi.org/10.12701/yujm.2019.00122
Injection of a local anesthetic through a vertebral artery can cause loss of consciousness and seizures [9].

RTF reportedly failed to detect 29.0% of intravascular injections compared to DSA during lumbosacral TFEB [10]. However, Kim et al. [11] found no benefit with use of DSA compared to RTF during lumbosacral TFEB. DSA has disadvantages such as increased radiation exposure to the physician and patient and high cost of equipment compared to RTF [12]. DSA for TFEB was reported to increase the effective radiation dose by 2.3- to 4.3-fold compared to conventional fluoroscopy [13].

To our knowledge, no report has prospectively compared DSA and RTF during TFEB at the whole spine level, including cervical, thoracic, lumbar, and sacral levels, in the same patient.

The present study investigated whether DSA improved the detection rate for intravascular injection during TFEB at the whole spine level, compared to that using RTF.

Materials and methods

1. Patients and exclusion criteria
The present study was approved by the Institutional Review Board of our hospital (DSMC 2015-09-042), and informed written consent was obtained from all participants.

We prospectively examined 316 TFEB procedures. Inclusion criteria were age over 18 years and radicular pain from herniated nucleus pulposus, spinal stenosis, post-surgical surgery syndrome; zoster-induced pain; or pain owing to other conditions such as complex regional pain syndrome. Exclusion criteria were pregnancy, allergy to contrast medium and local anesthetics, participant refusal, and persistent contraindication to epidural block such as coagulopathy and infection at the injection site.

2. Intervention and data collection
Two pain-management physicians were involved in this study. Both physicians were board-certified in the department of pain medicine, and had more than 8 years of working experience. TFEB was performed by one physician and simultaneously observed by the other physician.

Before the procedure, all participants were monitored with an electrocardiogram, pulse oximetry, and noninvasive blood pressure measurement. A 20- or 22-G cannula was inserted in the hand. The participants did not receive sedation. Under fluoroscopic guidance, TFEB was performed using a Quincke type, 25-G, 9-cm spinal needle (Taechang Industrial Co., Kongju, Korea). For cervical level injection, the participant was placed in a supine position on a table with the head slightly extended. The fluoroscope (Ziehm Vision, Ziehm Imaging, Nuremberg, Germany) was rotated obliquely 45-55° to the ipsilateral side to provide the best view of the selected neural foramen. The needle was advanced to the superior articular process, at the division between the caudal third and middle third. The needle was then advanced into the neural foramen, touching its posterior border to the halfway point between the medial and lateral borders of the articular pillars in an anteroposterior (AP) view. For thoracic level injection, the participant was placed in prone position. The fluoroscopic beam was aligned perpendicular to the vertebral endplates in an AP view and then rotated to a 10-20° oblique angle towards the side being injected. The needle was advanced from a point between the lateral margin of the pedicle and the medial aspect of the rib head to the posterior surface of the vertebral body using tunnel vision technique. For lumbar injections, the participant was placed in prone position. The fluoroscope was tilted in the caudocephalad direction to align parallel with the endplates in an AP view. The fluoroscope was rotated to a 20-30° oblique angle toward the side being injected, to bring the "Scotty dog" appearance of the spine into view. The needle was advanced into the neural foramen at a point just below the "chin" of the "Scotty dog" with tunnel vision technique. For sacral injections, the participant was placed in prone position. The fluoroscope was tilted in the caudocephalad direction to align parallel with the L5 inferior endplate and the S1 superior endplate in an AP view. The needle was advanced to the superior lateral quadrant of the neural foramen.

After confirmation of final needle position using biplanar fluoroscopy, 2 mL of nonionic contrast medium (Omnipaque 300, GE Healthcare, Little Chalfont, Buckinghamshire, UK) was injected at a rate of 0.5 mL/s under RTF; 30 s later, another 2 mL of nonionic contrast medium was injected at a rate of 0.5 mL/s under DSA. Intravascular injection was defined as contrast medium spreading out through the vascular channel during injection under RTF and DSA. If intravascular injection was observed, the needle position was changed. A total of 2 mL of 0.5% lidocaine mixed with dexamethasone 5 mg was injected after intravascular injection was ruled out.

3. Sample size
In a previous study, the incidence of intravascular injection during TFEB at the whole spine level was 10.5% [14]. We considered a 50% increase in the incidence of intravascular injection to be clinically important. The sample size was estimated with the requirement of <0.05 and <0.2 Type I and II error rates, respectively. Considering a 10% dropout rate, 316 TFEB cases in each group was required. A flow diagram of this study design is shown in Fig. 1.
Enrollment of 218 patients

Transforaminal epidural blocks
n=316

Detection of Intravascular injection using RTF
n=316

Positive on RTF
n=36

Negative on RTF
n=280

Detection of Intravascular injection using DSA
n=316

Positive on DSA (n=36)
Negative on DSA (n=0)

Positive on DSA (n=9)
Negative on DSA (n=271)

Intravascular injection present
n=36

Intravascular injection absent
n=0

Intravascular injection present
n=9

Intravascular injection absent
n=271

Fig. 1. Flow diagram of the study design. DSA, digital subtraction angiography; RTF, real-time fluoroscopy.

4. Statistical analysis
Data on the age, sex, diagnosis, spinal level, and procedure side were collected. The data were analyzed with McNemar’s test, using SAS software version 9.3 (Cary, NC, USA). The influence of factors associated with intravascular injection during TFEB was examined using logistic regression analysis, and the adjusted odds ratio (OR) and 95% confidence interval (CI) were also calculated. A p-value <0.05 was considered statistically significant.

Results
In total, 316 TFEB treatments were performed, with 56 injections (17.7%) at cervical levels, 31 (10.0%) at thoracic levels, 135 (42.7%) at lumbar levels, and 94 (29.7%) at sacral levels. There were no complications associated with TFEB. The 316 TFEB treatments were performed in 218 enrolled participants, with a mean age of 62.1 years. The characteristics of study participants are presented in Table 1. TFEB treatments were performed from C3 to S2 spinal levels.

The incidence of intravascular injection at each level detected with DSA and RTF is presented in Table 2. Thirty-six intravascular injections (12 [21.4%] at cervical, 2 [6.5%] at thoracic, 11 [8.1%] at lumbar, and 11 [11.7%] at sacral levels) were detected, for an overall intravascular injection rate of 11.4% using RTF. Forty-five intravascular injections (14 [25%] at cervical, 3 [9.7%] at thoracic, 14 [10.4%] at lumbar, and 14 [14.9%] at sacral levels) were detected, for an overall intravascular injection rate of 14.2% using DSA. The intravascular injection detection rate using DSA was statistically different from that using RTF (p=0.004). All intravascular injections detected using RTF were also observed using DSA. RTF missed 9 cases of intravascular injection (2 at cervical, 1 at thoracic, 3 at lumbar, and 3 at sacral levels) that were detected using DSA (RTF sensitivity, 80.0%).

Table 3 shows the adjusted OR and 95% CI for each variable during intravascular injection using DSA. Only the spinal level showed a significant association with intravascular injection. The incidence of intravascular injection was significantly higher at the cervical level than at the thoracic (p=0.009) and lumbar (p=0.011) levels. Patient age, sex, height, weight, procedure side, and diagnosis had no effect on the incidence.

Discussion
Our results indicate that intravascular injection was sequentially detected using RTF and DSA during TFEB at the whole spine.

Table 1. Characteristics of 316 injections performed in 218 participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>62.14±12.23</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161.81±8.32</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.70±9.83</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>155/161</td>
</tr>
<tr>
<td>Site of injection (right/left)</td>
<td>157/159</td>
</tr>
<tr>
<td>Level</td>
<td></td>
</tr>
<tr>
<td>Cervical</td>
<td>56</td>
</tr>
<tr>
<td>Thoracic</td>
<td>31</td>
</tr>
<tr>
<td>Lumbar</td>
<td>135</td>
</tr>
<tr>
<td>Sacral</td>
<td>94</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>Herniated nucleus pulposus</td>
<td>87</td>
</tr>
<tr>
<td>Spinal stenosis</td>
<td>166</td>
</tr>
<tr>
<td>Post-spinal surgery syndrome</td>
<td>21</td>
</tr>
<tr>
<td>Zoster-induced pain</td>
<td>31</td>
</tr>
<tr>
<td>Others</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>316</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation or number (proportion).
The overall incidence of intravascular injection was 14.2% using DSA and 11.4% using RTF. Nine intravascular injections were missed (2 at cervical, 1 at thoracic, 3 at lumbar, and 3 at sacral levels) with RTF compared to DSA. A 25% improvement was observed using DSA compared to RTF. Therefore, DSA had a better detection rate for intravascular injection during TFEB.

DSA is a radiological technique that can be used to clearly visualize and distinguish blood vessels from surrounding tissues; this is done by subtracting the pre-contrast image from the post-contrast injection image\[12,15\]. Visnjevac et al.\[16\] reported the efficacy of DSA in detection of intravascular penetration compared with RTF during TFEB in a recent meta-analysis. They included 1,290 TFEB cases (3.2% at cervical, 76.3% at lumbar, and 20.5% at sacral levels) and demonstrated that DSA showed a 32% improvement in detection of intravascular penetration during TFEB, compared to that using RTF. However, only 3.2% of cases in their study were performed at cervical levels and did not include any thoracic cases. Therefore, their study could not represent TFEB at the whole spine level. In contrast, the present study attempted to include more cervical and thoracic TFEB cases (17.7% at cervical and 10.0% at thoracic levels).

Even though previous studies demonstrated DSA to be superior to RTF for vascular detection during TFEB, McLean et al.\[15\] indicated that the main vascular uptake observed using DSA was

| Table 2. Incidence of intravascular injections during transforaminal epidural block |
|---------------------------------|---------------------------------|---------------------------------|
|                                 | Number of intravascular injections on DSA (%) | Number of intravascular injections on RTF (%) |
| Sex (n)                         | 21 (13.5)                                    | 17 (11.0)                                    |
| Male (155)                      |                                             |                                              |
| Female (161)                    | 24 (14.9)                                    | 19 (11.8)                                    |
| Level of injection (n)          | 14 (25.0)                                    | 12 (21.4)                                    |
| Cervical (56)                   |                                             |                                              |
| Thoracic (31)                   | 3 (9.7)                                      | 2 (6.5)                                      |
| Lumbar (135)                    | 14 (10.4)                                    | 11 (8.1)                                     |
| Sacral (94)                     | 14 (14.9)                                    | 11 (11.7)                                    |
| Side of injection (n)           | 22 (14.0)                                    | 18 (11.5)                                    |
| Right (157)                     |                                             |                                              |
| Left (159)                      | 23 (14.5)                                    | 18 (11.3)                                    |
| Diagnosis (n)                   | 10 (11.5)                                    | 9 (10.3)                                     |
| Herniated nucleus pulposus (87) |                                             |                                              |
| Spinal stenosis (166)           | 25 (15.1)                                    | 19 (11.4)                                    |
| Post-spinal surgery syndrome (21)| 5 (23.8)                                    | 5 (23.8)                                     |
| Zoster–induced pain (31)        | 3 (9.7)                                      | 2 (6.5)                                      |
| Others (12)                     | 2 (16.7)                                      | 1 (8.3)                                      |
| Total (316)                     | 45 (14.2)                                    | 36 (11.4)                                    |

Values are presented as number (%).

DSA, digital subtraction angiography; RTF, real-time fluoroscopy.

Table 3. OR and 95% CI of variables on intravascular penetration using DSA

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1.26</td>
<td>0.90-1.82</td>
</tr>
<tr>
<td>Age</td>
<td>0.99</td>
<td>0.96-1.01</td>
</tr>
<tr>
<td>Height</td>
<td>1.00</td>
<td>0.97-1.04</td>
</tr>
<tr>
<td>Weight</td>
<td>1.02</td>
<td>0.99-1.05</td>
</tr>
<tr>
<td>Side of injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>0.94</td>
<td>0.50-1.76</td>
</tr>
<tr>
<td>Level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical</td>
<td>2.88(a)</td>
<td>1.27-6.54</td>
</tr>
<tr>
<td>Thoracic</td>
<td>0.93</td>
<td>0.25-3.44</td>
</tr>
<tr>
<td>Lumbar</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Sacral</td>
<td>1.51</td>
<td>0.69-3.34</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herniated nucleus pulposus</td>
<td>1.21</td>
<td>0.31-4.73</td>
</tr>
<tr>
<td>Spinal stenosis</td>
<td>1.66</td>
<td>0.47-5.86</td>
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<tr>
<td>Post-spinal surgery syndrome</td>
<td>3.11</td>
<td>0.65-14.85</td>
</tr>
<tr>
<td>Zoster–induced pain</td>
<td>1.87</td>
<td>0.27-12.85</td>
</tr>
<tr>
<td>Others</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

\(a\) OR, odds ratio; CI, confidence interval; DSA, digital subtraction angiography.

https://doi.org/10.12701/yujm.2019.00122
attributed to venous uptake and that DSA did not increase the detection rate of arterial uptake. Moreover, in the study of Lee et al. [10], DSA did not successfully distinguish between arterial and venous injections.

Using RTF, Nahm et al. [14] conducted a large prospective study to evaluate the risk factors associated with intravascular injection in TFEB. They evaluated 2,145 injections performed on 1,088 patients and found a significant difference between cervical (20.6%) and sacral (16.5%) level injections and between lumbar (6.1%) and thoracic (8.2%) level injections. They also found no association between intravascular injection during TFEB and patient characteristics such as age, sex, body mass index, and diagnosis. No prior report has investigated the risk factors associated with intravascular injection using DSA at the whole spine level under various conditions. In the present study, the spinal procedure level was the only significant association identified, consistent with the results of a previous study using RTF [14]. The cervical level (25%) showed a significantly higher intravascular injection rate than the thoracic (9.7%) and lumbar (10.4%) levels.

The present study also found no difference in intravascular injection rates during TFEB according to the diagnosis, consistent with the results of a previous study using RTF [11,14]. We also found no differences in intravascular injection rates between DSA and RTF according to diagnosis.

In this study, we used a Quincke needle which is sharp. Because blunt needles will displace and not penetrate vessels owing to their dull tip [17,18], the use of blunt needles during TFEB has been suggested to avoid intravascular injection of steroids [7,19]. Animal studies have shown a reduced incidence of arterial puncture and bleeding with the use of blunt needles compared to that using sharp needles [17,18]. Several studies have found that use of blunt needles during lumbar TFEB could reduce intravascular injections and paresthesia compared to those using sharp needles [20-22]. However, Smuck et al. [23] failed to find any benefit with use of blunt needles during lumbosacral TFEB compared to that using sharp needles. This issue remains controversial, and further study is needed to determine whether the use of blunt needles could reduce intravascular injections during TFEB.

Intravascular injection was sequentially detected using RTF and DSA guidance in the present study. This study had some limitations. First, the procedural physician was not blinded to the type of imaging modality used to detect intravascular injection. To minimize confirmation bias and provide homogeneous procedural conditions for TFEB, the same procedural physician performed all 316 injections. Second, RTF and DSA were successively used in the same patient during TFEB. Intravascular injections detected by RTF were also detected by DSA. However, the injected contrast medium and extravasation of blood from intravascular penetration during RTF observation may have affected the detection of intravascular injection during DSA observation. Third, it may be impossible to define a vascular contrast spreading pattern as venous or arterial during epidural TFEB because these patterns were ambiguous despite the use of DSA [24,25]. We also could not differentiate between the two vascular patterns. Fourth, the proportions of cervical, thoracic, and lumbar levels in TFEB differed, as these reflected the distribution of patients with spinal pain in the pain clinic. Fifth, structural differences according to vertebral levels or individual spinal disease could affect the results, but further studies are needed to clarify this.

During TFEB at the whole spine level, DSA showed a better detection rate than RTF for intravascular injection. Special attention is recommended for cervical TFEB, which showed an intravascular injection rate that was significantly higher than that at other levels.

**Conflicts of interest**

No potential conflicts of interest relevant to this article was reported.

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**References**


Clinical significance of lymph node size in locally advanced cervical cancer treated with concurrent chemoradiotherapy

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Background: This study aimed to assess the in-field lymph node (LN) failure rate according to LN size and to investigate effect of LN size on the survival outcome of patients with locally advanced cervical carcinoma treated with concurrent chemoradiotherapy (CCRT).

Methods: A total of 310 patients with locally advanced cervical carcinoma treated with CCRT were enrolled in retrospective study. LN status was evaluated by magnetic resonance imaging. All patients received conventional external beam irradiation and high-dose rate brachytherapy, and concurrent cisplatin-based chemotherapy. In-field LN failure rate according to LN size was analyzed.

Results: The median follow-up period was 83 months (range, 3-201 months). In-field LN failure rate in patients with pelvic LN size more than 10 mm was significantly higher than that in patients with pelvic LN size less than 10 mm (p<0.001). A similar finding was observed in the in-field para-aortic LN (PALN) failure rate (p=0.024). The pelvic and PALN size (≥10 mm) was a significant prognostic factor of overall-survival (OS) and disease-free survival rate in univariate and multivariate analyses. The OS rate was significantly different between groups according to LN size (<10 mm vs. ≥10 mm).

Conclusion: A LN of less than 10 mm in size in an imaging study is controlled by CCRT. On the other hand, in LN of more than 10 mm in size, the in-field LN failure rate increase and the prognosis deteriorate. Therefore, a more aggressive treatment strategy is needed.

Keywords: Chemoradiotherapy; Lymph node; Uterine cervical neoplasms
clinical significance in patients who do not have local failure. The staging of cervical cancer follows the International Federation of Gynecology and Obstetrics (FIGO) stage system based on clinical staging. Although LN metastasis serves an important role in prognosis, LN status is not included in the staging [8]. In general, the larger the size of the primary tumor, the greater the dose required for achieving tumor control [9-11]. Similarly, the dose required to achieve local control of a metastatic LN increases with the size of the metastasis. The larger the size of the LN, the poorer the prognosis and local control rate. In many institutions, external beam boost irradiation has been used empirically for large metastatic LNs. However, there is limited clinical data for external beam irradiation to metastatic LNs [12-15], and the association between the size of the metastatic LN and LN control in CCRT remains to be fully elucidated. Previous studies included mixed groups comprising patients treated with CCRT or radiotherapy alone, which is inadequate to validate the effect of CCRT. Therefore, we aimed to assess the in-field LN failure rate according to LN size and to investigate the effect of LN size on the survival outcome in patients with cervical cancer treated with CCRT.

**Materials and methods**

**1. Patients**

This retrospective study was approved by the Institutional Review Board in Daegu Catholic University Medical Center (IRB No. CR-17-045-L). The medical records of 335 patients with cervical cancer treated with CCRT at the Daegu Catholic University Medical Center between 2000 and 2016, were reviewed. The inclusion criteria were as follows: 1) newly diagnosed histologically proven squamous cell carcinoma, adenocarcinoma, or adenosquamous carcinoma of the uterine cervix; 2) treatment using platinum-based CCRT; and 3) clinical and radiologic FIGO stage IB-IVA with no other evidence of distant metastasis. Of 335 patients, 25 patients were excluded for the following reasons: 1) surgical intervention prior to CCRT (n=20); and 2) incomplete treatment (n=5). The remaining 310 patients were included in the analysis. LN metastasis was evaluated by magnetic resonance imaging (MRI).

A total of 142 patients (45.8%) had metastatic pelvic LNs and 17 patients (5.5%) had metastatic PALNs. To investigate the effect of LN size on the treatment outcome, the patients were divided based on the LN size regardless of MRI evaluation results: those who had pelvic LN size less than 10 mm (n=196), those who had pelvic LN size from 10 mm to 19.99 mm (n=90), and those who had pelvic LN size 20 mm or more (n=24). Further, 189 patients had PALN size less than 5 mm, 108 patients had PALN size from 5 mm to 9.99 mm and 13 patients had PALN size 10 mm or more.

**Table 1. The characteristics of the enrolled patients**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr, mean±SD)</td>
<td>53.0±11.4</td>
</tr>
<tr>
<td>Pretreatment hemoglobin (g/dL, mean±SD)</td>
<td>11.7±1.62</td>
</tr>
<tr>
<td>Pretreatment SCC Ag. level (ng/mL, mean±SD)</td>
<td>9.9±16.1</td>
</tr>
<tr>
<td>Pathology</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>257 (82.9)</td>
</tr>
<tr>
<td>Adenocarcinoma or ASC</td>
<td>53 (17.1)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>IB1</td>
<td>53 (17.1)</td>
</tr>
<tr>
<td>IB2</td>
<td>46 (14.8)</td>
</tr>
<tr>
<td>IIA1</td>
<td>20 (6.5)</td>
</tr>
<tr>
<td>IIA2</td>
<td>12 (3.9)</td>
</tr>
<tr>
<td>IIIB</td>
<td>122 (39.4)</td>
</tr>
<tr>
<td>IIIA</td>
<td>2 (0.6)</td>
</tr>
<tr>
<td>IIIB</td>
<td>46 (14.8)</td>
</tr>
<tr>
<td>IVA</td>
<td>9 (2.9)</td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>2 (0.6)</td>
</tr>
<tr>
<td>Moderately</td>
<td>286 (92.3)</td>
</tr>
<tr>
<td>Poorly</td>
<td>26 (8.4)</td>
</tr>
<tr>
<td>Pelvic LN metastasis</td>
<td>142 (45.8)</td>
</tr>
<tr>
<td>Pelvic LN size (mm, mean±SD)</td>
<td>8.9±7.1</td>
</tr>
<tr>
<td>Pelvic LN size (mm)</td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>196 (63.2)</td>
</tr>
<tr>
<td>10–19.99</td>
<td>90 (29.0)</td>
</tr>
<tr>
<td>≥20</td>
<td>24 (7.7)</td>
</tr>
<tr>
<td>PALN metastasis</td>
<td>17 (5.5)</td>
</tr>
<tr>
<td>PALN size (mm, mean±SD)</td>
<td>4.8±2.5</td>
</tr>
<tr>
<td>PALN size (mm)</td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>189 (61.0)</td>
</tr>
<tr>
<td>5–9.99</td>
<td>108 (34.8)</td>
</tr>
<tr>
<td>≥10</td>
<td>13 (4.2)</td>
</tr>
</tbody>
</table>

SD, standard deviation; SCC Ag, squamous cell carcinoma associated antigen; ASC, adenosquamous cell carcinoma; LVI, lymphovascular invasion; LN, lymph node; PALN, para-aortic LN.

**2. Evaluation of lymph node status**

The LN status was evaluated mainly by MRI. The LN status was assessed using a combination of size, shape, and internal architecture [16,17]. For determining the LN size, the short axis diameter of the largest LN was measured. At our institution, positron emission tomography/computed tomography (PET/CT) has been in use since 2005. For evaluating a metastatic LN,
PET/CT shows better accuracy than MRI. However, it cannot change the prognosis [18]. Therefore, we use mainly MRI rather than PET/CT for evaluating LN status.

3. Treatment
All patients were scheduled to receive combined external-beam radiotherapy (EBRT) and intracavitary brachytherapy (ICBT). Seventy-two patients received extended-field pelvic radiotherapy (EF-PRT), and the superior border was extended to encompass the PALN area. In the patients with PALN involvement (n=17), PALN irradiation was done. In patients with no evidence of PALN involvement (n=55), the decision to use EF-PRT was at the discretion of the radiation oncologist, balancing the risk of occult PALN metastases against the potential for increased acute and late toxicity. In EF-PRT, the superior border was extended to encompass PALN area according to the discretion of the radiation oncologist as follows: T12-L1 (n=20), L1-L2 (n=5), or L2-L3 (n=47) inter space. All patients received a median EBRT dose of 45 Gy (range, 39.6-54 Gy) at 1.7 Gy (in some EF-PRT cases only) or 1.8 Gy per fraction with whole pelvic radiotherapy (WPRT) or EF-PRT. After WPRT or EF-PRT, the boost irradiation of median 9 Gy (range, 5.4-23.4 Gy) given at 1.8 Gy or 2 Gy per fraction to LN regions that had significant evidence of carcinoma involvement or LN more than 10 mm on MRI findings, involved parametrium, or involved regions of the pelvic sidewall. In boost irradiation, three-dimensional conformal radiotherapy or intensity-modulated radiotherapy (IMRT) has been used since 2009. After adequate tumor regression, high-dose-rate ICBT was performed twice per week using an iridium-192 remote after-loading technique. The standard prescribed dose for each brachytherapy in our institution was 5.0 Gy to A-point in six fractions, twice weekly. The prescribed A-point dose was median 30 Gy (range, 15-36 Gy). The combined total dose from EBRT and ICBT was calculated using a linear quadratic model to determine the radiobiological equivalent dose in 2 Gy fractions (EQD2) (α/β=10) [19]. The median total prescribed EBRT EQD2 to pelvic LNs area and PALN area was 53.1 Gy (range, 44.25-69.03 Gy) and 44.25 Gy (range, 40.71-58.41 Gy). The median total prescribed A-point EQD2 (EBRT+ICBT) was 81.75 Gy (range, 69.36-105.70 Gy). The median overall irradiated time was 59 days (range, 45-133 days; interquartile range, 54-63 days).

All patients received radiotherapy and concurrent cisplatin-based chemotherapy. During radiotherapy, chemotherapy with weekly cisplatin (40 mg/m\(^2\) weekly for 6 weeks) was given to 200 patients. Two cycles of cisplatin-based combination chemotherapy with cisplatin plus 5-fluorouracil (5-FU), or cisplatin plus paclitaxel at 3 weeks intervals during external beam radiotherapy were given to 52 and 58 patients, respectively. Chemotherapy with cisplatin and 5-FU consisted of an intravenous infusion of 75 mg/m\(^2\) of cisplatin (day 1), followed by an intravenous infusion of 4,000 mg/m\(^2\) of 5-FU over a 96-hour period (days 2-5). One liter of normal saline was given before and after cisplatin, and mannitol was used to increase the urine output (day 1). Chemotherapy with cisplatin plus paclitaxel consisted of an intravenous infusion of 135 mg/m\(^2\) of paclitaxel (day 1), followed by an intravenous infusion of 75 mg/m\(^2\) of cisplatin (day 2).

4. Response evaluation and follow-up
All patients were subjected to routine post-CCRT surveillance with physical examination, cervicovaginal cytology, laboratory test (e.g., squamous cell carcinoma antigen), and imaging studies, including abdominopelvic CT, MRI, and PET/CT. After completion of CCRT, the patients were evaluated every 3 months for the first 2 years and every 6 months thereafter. Recurrence was diagnosed through physical examination and diagnostic imaging (contrast-enhanced CT, MRI, and/or PET/CT scans) [16,17] and was confirmed histologically via needle aspiration or excisional biopsy when possible.

5. End points and statistical methods
The primary endpoint was in-field LN failure rate according to the size of LNs, and the overall survival (OS) rate and disease-free survival (DFS) rate according to the size of LNs. LN failure within the irradiated region was considered an in-field LN failure. We calculated all occurrences from the date of diagnosis to the date of relapse or the last date of follow-up. Deaths from other cause were censored at the time of last follow-up.

Comparison of variables was based on the t-test. The survival analysis was based on the life-table method of Kaplan-Meier. Univariate analyses were performed with log-rank tests. The Cox proportional hazard model was used to construct a multivariate model to predict survival. p-values were the result of two-sided tests and p-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

1. Analysis of lymph node size
The total number of patients was 310. Patients were divided into four groups according to pelvic LN status and size evaluated by MRI. Of these, 168 patients had LNs that had no significant evidence of carcinoma involvement on MRI, and had a short axis of less than 10 mm (group 0). The other 142 patients had LNs with
evidence of heavily involved carcinoma on MRI, and these patients were further divided into three groups according to their LN size (group 1, <10 mm; group 2, 10-19.99 mm; group 3, ≥20 mm).

On evaluation the in-field failure rate for the PALN area, 72 patients who received EF-PRT were analyzed. These patients were divided into three groups according to their PALN size (group 0, <5 mm; group 1, 5-9.99 mm; group 2, ≥10 mm).

2. In-field pelvic lymph node failure rate
Due to the possibility of micro-metastasis of LN, the failure rate of all groups was analyzed. There was no significant difference between group 0 and group 1 (5-year in-field failure rate, 1.3% and 0%, respectively; 10-year in-field failure rate, 1.3% and 0%, respectively). The 5-year in-field failure rates among the patients in the groups 1, 2, and 3 were 0%, 9.6%, and 22.6%, respectively. The 10-year in-field failure rates among the patients in the groups 1, 2, and 3 were 0%, 12%, and 29%, respectively. There were statistically significant differences in the 5- and 10-year in-field failure rates between group 1 and group 2/3 (group 1 vs. group 2, 3, p<0.001; group 1 vs. group 3, p<0.001). The in-field failure rate in patients with LN size 10 mm or more was significantly increased. In addition, although there was no statistically significant difference, the in-field failure rate tended to increase as the size increased (group 2 vs. group 3, p=0.089). The cumulative in-field pelvic LN failure rate according to LN size is shown in Fig. 1.

3. In-field para-aortic lymph node failure rate
The 5-year in-field failure rates among patients in groups 0, 1, and 2 were 5.9%, 5%, and 18.5%, respectively, and 10-year in-field failure rates were 5.9%, 5%, and 45.7%, respectively. There were statistically significant differences between group 0/1 and group 2 (group 0/1 vs. group 2, p=0.024). Like the in-field failure rate for pelvic LN, the in-field failure rate for PALN was significantly increased with LN size 10 mm or more. The cumulative in-field PALN failure rate according to LN size in patients treated with EF-PRT is shown in Fig. 2.

4. Survival outcome: prognostic variables
The results of the univariate analysis showed that advanced stage (I/II vs III/IV), pretreatment hemoglobin (<12.3 g/dL), tumor size (≥ 4 cm), and LN size in the pelvic and para-aortic areas (≥10 mm) were significant factors of poor OS rate and DFS rate. The 10-year OS rate in patients with pelvic LN size <10 mm and ≥10 mm was 89.2% and 64.1%, respectively, and the 10-year OS rate in patients with PALN size <10 mm and ≥10 mm was 82.4% and 33.3%, respectively (Table 2). The OS rates were statistically different according to LN size 10 mm or more for both pelvic and PALNs (pelvic LN, p<0.001; PALN, p<0.001; Figs. 3, 4). The 10-year DFS rate in patients with pelvic LN size <10 mm and ≥10 mm was 83.3% and 57.3%, respectively, and the 10-year DFS rate in patients with PALN size <10 mm and ≥10 mm was 76.0%.
and 22.8%, respectively. Of these, pelvic and PALN size (≥10 mm) was a significant prognostic factor of OS and DFS rates in multivariate analysis (pelvic LN, \( p = 0.003 \); PALN, \( p = 0.033 \), Table 3). The irradiated dose was not significantly associated with poor OS and DFS rates.

### Discussion

Although standard radiotherapy regimens have been established for the treatment of primary cervical cancer, optimal radiotherapy regimens for regional LN metastases remain unclear, particularly for bulky LN [20,21]. The relationship between the size of metastatic LN and LN control in CCRT remains to be fully elucidated. In our study, LN size less than 10 mm was well-controlled, and the in-field failure rate for LN sizes ≥10 mm was increased. The in-field failure rate tended to increase as the LN size increased.

In the era of radiotherapy alone for advanced cervical cancer, Hacker et al. reported that the surgical removal of enlarged LNs prior to radiotherapy improves prognosis [22]. After the introduction of CCRT, since chemotherapy acts as a radiosensitizer, it may affect the control rate after bulky LN dissection, however, the results are insufficient. Lai et al. reported a study that assessed the prognostic significance of surgical staging in locally advanced cervical cancer [23]. The progression-free survival and OS rates of patients who underwent surgical staging were significantly poorer than those of patients who underwent clinical staging. It was suggested that the

<table>
<thead>
<tr>
<th>Table 2. Univariate survival analysis</th>
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<tbody>
<tr>
<td>Variable</td>
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<tr>
<td>-------------------------------------</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
</tr>
<tr>
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</tr>
<tr>
<td>≥50</td>
</tr>
<tr>
<td>Stage</td>
</tr>
<tr>
<td>I/II</td>
</tr>
<tr>
<td>III/IV</td>
</tr>
<tr>
<td>Pathologic type</td>
</tr>
<tr>
<td>SCC</td>
</tr>
<tr>
<td>AC/ASC</td>
</tr>
<tr>
<td>Primary tumor size (cm)</td>
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<td>&lt;4</td>
</tr>
<tr>
<td>≥4</td>
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<tr>
<td>Differentiation</td>
</tr>
<tr>
<td>Well/moderately</td>
</tr>
<tr>
<td>Poorly</td>
</tr>
<tr>
<td>LVI</td>
</tr>
<tr>
<td>Absent</td>
</tr>
<tr>
<td>Present</td>
</tr>
<tr>
<td>Pretreatment SCC Ag. (ng/mL)</td>
</tr>
<tr>
<td>&lt;4</td>
</tr>
<tr>
<td>≥4</td>
</tr>
<tr>
<td>Pretreatment hemoglobin</td>
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<td>Anemia(a)</td>
</tr>
<tr>
<td>Pelvic LN size (mm)</td>
</tr>
<tr>
<td>&lt;10</td>
</tr>
<tr>
<td>≥10</td>
</tr>
<tr>
<td>PALN size (mm)</td>
</tr>
<tr>
<td>&lt;10</td>
</tr>
<tr>
<td>≥10</td>
</tr>
</tbody>
</table>

OS, overall survival rate; DFS, disease-free survival rate; SCC, squamous cell carcinoma; AC, adenocarcinoma; ASC, adenosquamous cell carcinoma; LVI, lymphovascular invasion; SCC Ag, squamous cell carcinoma associated antigen; LN, lymph node; PALN, para-aortic lymph node.

\(a\)Hemoglobin <12.3 g/dL was considered as anemia.
of LN metastasis assessed by MRI, a wide range of sensitivity, specificity, and accuracy were reported; 30-73%, 93%, and 73% respectively [26,27]. In addition, it has been suggested that the probability of microscopic LN disease is 20-25%, even with no significant evidence of carcinoma involvement in an imaging study [28]. However, in our study, the in-field failure rate and OS differed only according to the LN size despite the limitation of clinical staging. For pelvic LNs, there was no significant difference between group 0 and group 1 (5-year in-field failure rate, 1.3% and 0%, respectively; 10-year in-field failure rate, 1.3% and 0%, respectively). Therefore, for an LN size of less than 10 mm, the surgical assessment of PALN status prior to CCRT was ineffective compared with the use of imaging techniques [23-25]. Because the benefits of surgical dissection and biopsy are unclear, in the present clinical setting, oncologists use a radiologic method for the evaluation of LN status in almost all cases of locally advanced cervical cancer.

Therefore, currently, it is universally accepted that CCRT be performed following imaging studies in patients with locally advanced cervical cancer. The preoperative PET/CT evaluation of LNs assists in identifying distant metastasis and PALN metastasis, but does not appear to improve survival rate. On evaluation of LN metastasis assessed by MRI, a wide range of sensitivity, specificity, and accuracy were reported; 30-73%, 93%, and 73%, respectively [26,27]. In addition, it has been suggested that the probability of microscopic LN disease is 20-25%, even with no significant evidence of carcinoma involvement in an imaging study [28]. However, in our study, the in-field failure rate and OS differed only according to the LN size despite the limitation of clinical staging. For pelvic LNs, there was no significant difference between group 0 and group 1 (5-year in-field failure rate, 1.3% and 0%, respectively; 10-year in-field failure rate, 1.3% and 0%, respectively). Therefore, for an LN size of less than 10 mm, the

### Table 3. Multivariate survival analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>RR</th>
<th>95% CI of RR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.011</td>
<td>0.983-1.039</td>
<td>0.456</td>
</tr>
<tr>
<td>Stage (I/II vs. III/IV)</td>
<td>0.55</td>
<td>0.267-1.132</td>
<td>0.105</td>
</tr>
<tr>
<td>Pathologic type (SCC vs. AC/ASC)</td>
<td>0.991</td>
<td>0.418-2.351</td>
<td>0.984</td>
</tr>
<tr>
<td>Primary tumor size</td>
<td>1.002</td>
<td>0.982-1.021</td>
<td>0.874</td>
</tr>
<tr>
<td>Differentiation (well/moderately vs. poorly)</td>
<td>0.563</td>
<td>0.197-1.609</td>
<td>0.283</td>
</tr>
<tr>
<td>LVI (present vs. absent)</td>
<td>0.872</td>
<td>0.349-2.178</td>
<td>0.769</td>
</tr>
<tr>
<td>Pretreatment SCC Ag. (&lt;4 vs. ≥4 ng/mL)</td>
<td>1.01</td>
<td>0.999-1.021</td>
<td>0.087</td>
</tr>
<tr>
<td>Pretreatment hemoglobin (normal vs. anemiaa)</td>
<td>0.994</td>
<td>0.838-1.179</td>
<td>0.946</td>
</tr>
<tr>
<td>Pelvic LN size (&lt;10 mm vs. ≥10 mm)</td>
<td>0.392</td>
<td>0.210-0.731</td>
<td>0.003</td>
</tr>
<tr>
<td>PALN size (&lt;10 mm vs. ≥10 mm)</td>
<td>0.402</td>
<td>0.174-0.927</td>
<td>0.033</td>
</tr>
</tbody>
</table>

RR, relative risk; CI, confidence interval; SCC, squamous cell carcinoma; AC, adenocarcinoma; ASC, adenosquamous cell carcinoma; LVI, lymphovascular invasion; SCC Ag, squamous cell carcinoma associated antigen; LN, lymph node; PALN, para-aortic lymph node.

*Hemoglobin <12.3 g/dL was considered as anemia.
rate of control is similar regardless of whether the MRI reveals have significant evidence of carcinoma involvement. Negative LNs that had no significant evidence of carcinoma involvement or LNs less than 10 mm in MRI findings showed good control over conventional radiation dose, regardless of the sensitivity. Not all enlarged LNs are metastatic; however, they have a high metastatic potential. In our study, the factor of LN size ≥10 mm was associated with an increase in the in-field failure rate and affected the survival outcome confirmed by multivariate analysis. Therefore, it is appropriate to use LN size measurement MRI in treatment planning.

There are several studies assessing the effects of LN size on prognosis. Studies have reported that an enlarged LN, based on 20 mm or 15 mm size, is associated with a poor prognosis [15,29,30]. Song et al. reported that the OS and DFS rate were poorer when the LN size was larger than 1.5 cm [15]. Since the study included mixed groups composed of patients treated with CCRT or radiotherapy alone, it is inadequate to validate the effect of CCRT. In the present study, the size of 10 mm, which is suspicious of imaging positive lymphadenopathy, was used as the most commonly used size criteria. As a result, it was confirmed that an LN size ≥10 mm affects the in-field failure rate and OS rate in a CCRT setting [31]. There was no significant difference in the in-field failure rate (p=0.068) or OS (p=0.525) when a pelvic LN ≥20 mm was compared to a pelvic LN size ≥10 mm. Therefore, it is reasonable to use 10 mm as a criterion for determining positive LN status.

Optimal treatment with CCRT for an enlarged LN in locally advanced cervical cancer remains controversial. Traditionally, doses of 45-50 Gy in conventional fractionation are delivered to the whole pelvis to treat cervical cancer with or without concurrent chemotherapy, primarily due to adjacent normal tissue tolerance as a limiting factor. Subsequent small field radiation boosts of 5.4-10 Gy in conventional fractionation are frequently administered to metastatic LNs. The interdigitation pelvic node boosts with brachytherapy can present with specific challenges. An anteroposterior-posteroanterior boost technique may be used if boost fields are small and if less than an additional 5.4-10 Gy is needed to increase the combined external beam and brachytherapy dose to a minimum of 60-66 Gy [20,21]. According to Toita et al., the prognosis was no poorer when the LN was irradiated with a dose less than that given using the conventional method [32]. Recently, Ariga et al. demonstrated that external beam boost irradiation to positive pelvic LNs achieves favorable nodal control without increasing late complications [13]. Hata et al. also reported the radiotherapy effectively controlled pelvic LN metastases in patients with cervical cancer with most LNs <24 mm in diameter controlled by the total dose of 50.4 Gy in 1.8 Gy fractions and radiation boost over 50.4 Gy may improve the control of metastatic LNs ≥24 mm, particularly with concurrent chemotheraphy [12]. They also suggested that higher doses to metastatic LNs may increase intestinal toxicities. In this CCRT study, the median total prescribed EBRT EQD2 to pelvic LNs area and PALN area was 53.1 Gy (range, 44.25-69.03 Gy) and 44.25 Gy (range, 40.71-58.41 Gy). It considered that in our study, pelvic LNs and PALNs that had evidence of heavily involved with carcinoma on MRI was given the dose as a traditional standard with concurrent chemotherapy. Despite the traditional standard dose in CCRT, our study showed that significantly higher incidence of in-field LN failure LNs recurrence in patients with pelvic LN size ≥10 mm, than that in patients with pelvic LN size <10 mm. Therefore, the development of more effective radiotherapy strategies is required to reduce the pelvic LN recurrence in patients with pelvic LN size ≥10 mm.

A higher dose than the traditional standard dose can be delivered using additional boost technique. Hata et al. reported that larger LNs that were >24 mm in diameter may require higher doses, up to about 55.8 Gy [12]. Rash et al. reported that the control rate was improved when a total dose of ≥54 Gy was delivered using a boost technique to treat pelvic and para-aortic lymphadenopathy [33]. However, results on the toxicity associated with higher radiation doses are insufficient. Normal tissue complication probability should be considered when increasing the radiation dose for achieving control of an enlarged LN. High-dose boost irradiation to enlarged LNs may increase the risk of high-dose exposure to the colon and small intestine due to their proximity to pelvic LNs. When higher boost doses are required, more complex techniques are recommended; however, to avoid compromising subsequent brachytherapy, care must be taken to minimize the dose to the bowel, rectum, and bladder from high-precision radiotherapy such as image-guided radiotherapy (IGRT) and IMRT. Recently, dose escalation studies have been performed using IMRT, volumetric modulated arc therapy, and IGRT; however, the problem of bowel toxicity remains, which limits the use of higher irradiation doses [34-36]. There are problems with the IMRT itself which are related to target definition, inter- and intra-fraction motion, and tumor regression during treatment [37,38]. However, some studies have shown that excellent control of the metastatic LNs with a median dose of 62 Gy (range, 59.4-64 Gy) using IMRT was achieved. Thus, we need to wait for the results of further randomized prospective trials and the result of long-term follow-up studies. Additionally, the use of high-precision radiotherapy such as image-guided stereotactic body radiotherapy or particle radiotherapy is expected to be beneficial for boost irradiation to enlarged LNs. By using these recent advanced treatment methods, higher doses can be delivered to the tumor without increasing...
doses to adjacent normal tissue.

A limitation of the present study includes its retrospective study design, which may be affected by selection bias. The LN dose by ICBT was not analyzed as image-guided brachytherapy was not performed, and irradiation dose to pelvic LNs may have been underestimated. There was a relatively small number of patients with PALNs of ≥10 mm. Therefore, caution is required when ascribing clinical meaning to the results of the present study. The results of the present study must be validated on a larger patient cohort and further prospective randomized investigations of radiation dose escalation with IMRT are required to decrease pelvic LN recurrence.

In conclusion, the present study demonstrates that an LN of less than 10 mm in size in an imaging study was controlled by CCRT. On the other hand, in CCRT with boost irradiation to LNs of size ≥10 mm, the in-field failure rate increases, and the prognosis deteriorates. Currently, treatment guidelines for enlarged LNs remain unclear; therefore, a more aggressive treatment strategy to overcome the adverse effects of enlarged LNs on survival outcomes is required.

Acknowledgements

This work was supported by research grants from the Catholic University of Daegu in 2017.

Conflicts of interest

No potential conflicts of interest relevant to this article was reported.

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References

15. Song S, Kim JY, Kim YJ, Yoo HJ, Kim SH, Kim SK, et al. The size of the metastatic lymph node is an independent prognostic
What are the most important prognostic factors in patients with residual rectal cancer after preoperative chemoradiotherapy?

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Introduction

Preoperative chemoradiotherapy (CRT) followed by principle of total mesorectal excision (TME) is now become the standard treatment for clinically staged, locally advanced mid- or low-rectal cancer, i.e. tumor with extension of cT3-T4 and/or the presence of suspected regional metastatic lymph nodes [1]. Preoperative CRT may facilitate complete radical resection negative surgical resection margin and sphincter preservation with by downsize and/or downstage of primary tumor [2], and allow an evaluation of chemo- or radio-sensitivity of tumor against the given therapeutic regimen [3]. Furthermore, the degree of response to preoperative CRT is regarded a prognostic indicator of patient outcome, and the patients achieving pathological complete response (pCR) after preoperative CRT have been showed excellent disease-free survival (DFS) [3]. However, pCR rate

Keywords: Circumferential resection margin; Preoperative chemoradiotherapy; Prognosis; Rectal cancer; Stage; Tumor regression grade

Background: We aimed to establish robust histoprognostic predictors on residual rectal cancer after preoperative chemoradiotherapy (CRT).

Methods: Analyzing known histoprognostic factors in 146 patients with residual disease allows associations with patient outcome to be evaluated.

Results: The median follow-up time was 77.8 months, during which 59 patients (40.4%) experienced recurrence and 41 (28.1%) died of rectal cancer. On univariate analysis, residual tumor size, ypT category, ypN category, ypTNM stage, downstage, tumor regression grade, lymphatic invasion, perineural invasion, venous invasion, and circumferential resection margin (CRM) were significantly associated with recurrence free survival (RFS) or/and cancer-specific survival (CSS) (all \(p<0.005\)). On multivariate analysis, higher ypTNM stage and CRM positivity were identified as independent prognostic factors for RFS (ypTNM stage, \(p=0.024\); CRM positivity, \(p<0.001\)) and CSS (\(p=0.022\), \(p=0.017\), respectively). Furthermore, CRM positivity was an independent predictor of reduced RFS and CSS, irrespective of subgrouping according to downstage (non-downstage, \(p<0.001\) and \(p<0.001\); downstage, \(p=0.002\) and \(p=0.002\)) or lymph node metastasis (non-metastasis, \(p<0.001\) and \(p=0.001\); metastasis, \(p<0.001\) and \(p<0.001\)).

Conclusion: CRM status may be as powerful as ypTNM stage as a prognostic indicator for patient outcome in patients with residual rectal cancer after preoperative CRT.

Keywords: Circumferential resection margin; Preoperative chemoradiotherapy; Prognosis; Rectal cancer; Stage; Tumor regression grade
has been reported in 8-27% of patients receiving preoperative CRT [4-6], and the majority of patients were not achieved pCR. Therefore, it is great importance to identified prognostic factors of patients with residual rectal cancer after preoperative CRT.

Pathological prognostic factors, such as ypTNM stage, perineural invasion, lymphatic invasion, venous invasion, circumferential resection margin (CRM), and tumor regression grade (TRG), are easily identified but are still important for patient outcome and essential to determining further clinical management of patients [1,2,7-9]. Several studies investigated the association between pathological factors and patient outcome in rectal cancer patients treated with preoperative CRT, however, data from the previous studies on the prognostic value of pathological factor are inconsistent [1,2,10-12]. Quah et al. [2] demonstrated that "pathologic stage (ypTN stage)" was the most precise prognostic predictor. On the other hand, Gosens et al. [11] reported that CRM positivity and pathologic lymph node status were most significant factors and their combination might give stronger evidence for patient outcome than classic ypTNM stage. In addition, Huebner et al. [12] showed that TRG and pathologic lymph node status were relevant prognostic predictors. However, data from other studies on the prognostic value of TRG in rectal cancer patients received preoperative CRT are inconsistent [2,9,12-14]. Several factors could be responsible for these discrepancies, including inter-observer variation and different methods of quantifying for tumor regression. Unfortunately, most studies evaluated the prognostic significance of pathological factors in rectal cancer patients treated with preoperative CRT without consideration of pCR. Because patients achieved pCR have all favorable histoprognostic status, including absence of lymphatic invasion, perineural invasion, and venous invasion, negative CRM, ypT0N0 (by seventh Union for International Cancer Control [UICC]/American Joint Committee on Cancer [AJCC] [15]), prognostic value of analyzing proven pathological factors could be different in patients with residual disease after preoperative CRT. Therefore, robust pathological prognostic factors of residual disease will also need to be determined.

In this study, we investigated known histoprognostic factors in correlation with patient survival to identify robust prognostic predictors in patients with residual disease after preoperative CRT.

Materials and methods

1. Patient population
After the approval of the Institutional Review Boards of Kyungpook National University Medical Center, medical records of 146 patients with rectal cancer who underwent preoperative CRT followed by TME at Kyungpook National University Medical Center between January 2006 and December 2011 were first corrected (IRB No: 2014-04-215). Patients with clinical evidence of distant metastases at the time of diagnosis and patients who were reported as pCR were excluded. All patients had a biopsy-proven diagnosis of primary rectal adenocarcinoma and were identified locally advanced tumors (cT3-4 or cN+). Hematoxylin and eosin (H&E)-stained slides and formalin-fixed, paraffin-embedded (FFPE) tumor samples from surgically resected specimens for each case were retrospectively retrieved.

2. Preoperative chemoradiotherapy and surgery
All patients received preoperative long-course radiotherapy (45 or 50 Gy over 5 weeks) plus 5-fluorouracil-based concomitant chemotherapy (intravenous bolus or continuous infusion) during radiotherapy. All patients were performed radical resection, according to the principle of TME, between 6 and 8 weeks after the completion of preoperative CRT. The detailed process of preoperative CRT followed by surgical protocol has been previously published [16,17].

3. Clinical assessment
After surgical resection, patients were followed 3-month intervals for the first 2 postoperative years and 6-month intervals thereafter. The clinical workup included digital rectal examination, serum carcinoembryonic antigen (CEA), colonoscopy, computed tomography/CT of the abdomen and pelvis or pelvic magnetic resonance imaging, and whole-body positron emission tomography/CT [16].

The recurrence free survival (RFS) was calculated from the time of surgery until the time of any recurrence of rectal cancer, and the cancer-specific survival (CSS) was calculated from the date of surgery to the date of death caused by rectal cancer or the date of last contact, as applicable.

4. Pathological assessment and tumor regression grade
For each surgically resected specimen, tumor size and distance of tumor from distal resection margin were measured at the time of gross examination. The non-peritonealized surface of the surgical specimen was painted with green ink to facilitate recognition of CRM. The specimen was opened along the anterior aspect and fixed in 10% neutral-buffered formalin overnight [18]. The entire tumor site was serially sectioned and embedded in paraffin, and 4-μm sections were cut and stained with H&E [1]. All H&E-stained slides were retrospectively reviewed by two pathologists (A.N.S and G.S.Y) blinded to the patients’ clinical data and re-evaluated pathological characteristics, as follows: tumor size;

https://doi.org/10.12701/yujm.2019.00157
presence of lymphatic invasion, perineural invasion, and venous invasion; ypT stage, ypN stage, and ypTNM stage; TRG; and CRM. The tumors stage was determined according to the UICC/AJCC staging manual for colorectal cancer (7th edition) [15]. Tumor regression of the primary tumor against preoperative CRT was semi-quantitatively assessed using 5-point TRG system as initially described by Rödel et al. [3], as follows: grade 0, no regression; grade 1, minor regression (<25% of tumor mass); grade 2, moderate regression (≥25% to 50% of tumor mass); grade 3, good regression (>50% of tumor mass); and grade 4 (complete remission or pCR). A tumor with acellular mucin pools in whole tissue was considered as pCR and was excluded in the present study. In patients with non-pCR, inter-observer reproducibility for TRG was analyzed. Final consensus in cases showing discrepancies for TRG was reached in common session using the multi-head microscope. The CRM was measured as the shorter distance from the outermost part of the tumor cells to the inked resection margin. To evaluate the exact length, the CRM was measured by a ruler or a microscope graticule, and CRM positivity was defined as ≤1 mm [19]. If cases with CRMs of 1 to 2 mm were found, additional multiple-level serial and deep sections were examined in these cases to detect CRM positivity by hidden malignancy.

5. **KRAS and BRAF mutation**
Deoxyribonucleic acid (DNA) was extracted from FFPE representative tumor tissues using Maxwell 16FFPE Tissue LEV DNA Purification kit (Promega, Seoul, Korea) and identified for the presence of mutations in KRAS exon 2 (codon 12 and 13) and in BRAF V600E using the PNAClamp™KRAS and PNAClamp™BRAF mutation Detection kit (Panagene, Daejeon, Korea) in PNA-mediated real-time polymerase chain reaction (PCR) as described previously [20]. The efficiency of PCR clamping was calculated by measuring the threshold cycle (Ct) value. Ct values for control and mutation assays were automatically evaluated from SYBR Green amplification plots and delta-Ct values were calculated (control Ct – sample Ct). A higher delta-Ct value shows that the mutant was efficiently amplified. The cut-off delta-Ct was defined as 2 for KRAS and BRAF mutation.

6. **Statistical analyses**
All statistical analyses were performed using IBM SPSS version 19.0 (IBM Co., Armonk, NY, USA). To evaluate the inter-observer agreement for TRG, Cohen’s Kappa (κ) was used. Significant differences for survival between the two groups were compared using the log-rank test, and survival curves were plotted using the Kaplan-Meier method. Multivariate analyses were performed using the Cox proportional hazard regression model to verify the independent prognostic impact for each factor. For multivariate analyses, covariates that were proven to be significant in the univariate analysis were controlled, and any possibility of multicollinearity was excluded from the final model. The hazard ratio (HR) and 95% confidence interval (CI) were assessed for each factor. All tests were two-sided, and statistical significance was considered as p<0.05.

**Results**

1. **Patients’ clinicopathologic characteristics**
A total of 146 patients with residual disease in the surgically resected rectal cancer after preoperative CRT were analyzed. The baseline characteristics of patients are provided in Table 1. The patient group included 102 (69.9%) male and 44 (30.1%) female with median age of 60.0 years (range, 29-85 years). The majority of patients were identified tumor distance from anal verge of <5 cm (76.7%) and pretreatment serum CEA level of ≤5 ng/mL (66.9%). At the time of initial diagnosis, 17 (11.6%) patients and 129 (88.4%) had evidence of cTNM stage II and III, respectively.

On gross examination, residual tumor size was ranged from 2.3 to 10.0 cm (median range, 6.0 cm). At the time of surgical resection, 31 tumors (21.2%) were ypT stage I, 68 tumors (46.6%) were stage II, and 47 tumors (32.2%) were stage III. When comparing with cTNM stage, 92 (63.0%) patients were experienced downstage after preoperative CRT. For the degree of response to preoperative CRT, TRG 0 was found in 4 of 146 (2.7%) patients, TRG 1 in 21 (14.4%), TRG 2 in 55 (37.7%), and TRG 3 in 66 (45.2%). All cases were independently re-evaluated TRG by two pathologists, and inter-observer agreement was analyzed. When comparing an each TRG, the inter-observer agreement showed a moderate agreement (κ=0.508; p<0.001), whereas, when comparing dichotomous groups (TRG 0&1 vs. TRG 2&3), kappa value reached 0.534 (p<0.001). In addition, CRM positivity was observed in 30 of 146 (20.5%) patients. CRM positivity was more commonly identified in adverse ypT category tumors (p=0.001), adverse ypTNM stage tumors (p=0.002), tumors with lower TRG, and tumors with perineural invasion (p=0.058). Of the 146 patients, mutation testing was available for 105 patients (72.4%), 29 (27.6%) and 4 (3.8%) were identified to have KRAS mutant and BRAF mutant primary tumors, respectively.

2. **Prognostic factors and univariate analyses**
At the time last analyses (December 2017), the median follow-up time was 77.8 months (range, 3.1-141.7 months), during which 59 patients (40.4%) experienced recurrence and 41 (28.1%) died.
In univariate analysis, the following 10 factors were identified as being significantly associated with shorter RFS or/and CSS: larger residual tumor size; higher ypT category, ypN category, and ypTNM stage; non-downstage after preoperative CRT; lower TRG; presence of lymphatic invasion, perineural invasion, and venous invasion; and CRM positivity (all \( p < 0.005, \) Table 2; Fig. 1). Unfortunately, KRAS mutation-positivity and BRAF mutation-positivity were not associated with RFS (KRAS, \( p = 0.107; \) BRAF, \( p = 0.435 \)) and CSS (KRAS, \( p = 0.122; \) BRAF, \( p = 0.186 \)). Then, we performed subgroup analyses according to downstage and lymph node metastasis (LNM) status after preoperative CRT, respectively. As shown Table 3, in patients subgroup showing non-downstage (n=54), more advanced ypT category, lower TRG, presence of lymphatic invasion, perineural invasion, and venous invasion, and CRM positivity (Figs. 2A, 2B) were related to worse RFS or CSS, and both. In patients subgroup showing downstage (n=92), larger residual tumor size, higher ypT category, and CRM positivity (Figs. 2C, 2D) were correlated with poorer RFS or/and CSS. On the other hand, in patients without LNM (n=99), larger residual tumor size, higher ypT category, and CRM positivity (Figs. 2E, 2F) were associated with shorter RFS or CSS, and both (Table 3). In patients with LNM (n=47), more advance ypT category, lower TRG, presence of perineural invasion and venous invasion, and CRM positivity (Figs. 2G, 2H) correlated with worse RFS or/and CSS (Table 3).

### 3. Multivariate analyses

As shown Table 4, multivariate analyses revealed the following independent unfavorable prognostic factors for RFS: larger residual tumor size (HR=1.753; \( p = 0.049 \)), CRM positivity (HR=3.613; \( p < 0.001 \)), and presence of venous invasion (HR=20.425; \( p < 0.001 \)); and for CSS: higher ypTNM stage (HR=3.413; \( p = 0.004 \)), larger residual tumor size (HR=2.371; \( p = 0.020 \)) presence of perineural invasion (HR=2.634; \( p = 0.009 \)), and CRM positivity (HR=4.133; \( p < 0.001 \)).

Then, we also evaluated the independency of prognostic factors within aforementioned subgroups. In patients with non-downstage, for RFS, presence of venous invasion (HR=18.066; \( p < 0.001 \)) and CRM positivity (HR=3.715; \( p = 0.004 \)); for CSS, presence of venous invasion (HR=4.072; \( p = 0.023 \)), perineural invasion (HR=3.067; \( p = 0.023 \)), and CRM positivity (HR=2.666; \( p = 0.043 \)) were independent negative prognostic factors, respectively. While, in patients with downstage, for RFS, CRM positivity (HR=2.388; \( p = 0.028 \)) for CSS, larger residual tumor size (HR=3.599; \( p = 0.028 \)) and CRM positivity (HR=3.829; \( p = 0.019 \)) were independent adverse prognostic factors, respectively. Additionally, in patients without lymph node

### Table 1. Characteristics of the patients with residual rectal cancer after preoperative CRT (n=146)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (yr)</td>
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<td></td>
</tr>
<tr>
<td>Median</td>
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<td></td>
</tr>
<tr>
<td>Range</td>
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<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>102</td>
<td>69.9</td>
</tr>
<tr>
<td>Female</td>
<td>44</td>
<td>30.1</td>
</tr>
<tr>
<td>Tumor distance from anal verge (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>112</td>
<td>76.7</td>
</tr>
<tr>
<td>( \geq 5 )</td>
<td>34</td>
<td>23.3</td>
</tr>
<tr>
<td>Residual tumor size (cm)</td>
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<tr>
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<td>Preoperative CEA (ng/mL)(^\text{a})</td>
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<tr>
<td>( \leq 5 )</td>
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<td>&gt;5</td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>37.7</td>
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<td>3</td>
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<tr>
<td>I</td>
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</tr>
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<td>II</td>
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<td>III</td>
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<tr>
<td>No</td>
<td>54</td>
<td>37</td>
</tr>
<tr>
<td>Yes</td>
<td>92</td>
<td>63</td>
</tr>
<tr>
<td>KRAS mutation(^\text{a})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>76</td>
<td>72.4</td>
</tr>
<tr>
<td>Positive</td>
<td>29</td>
<td>27.6</td>
</tr>
<tr>
<td>BRAF mutation(^\text{a})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>101</td>
<td>96.2</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>3.8</td>
</tr>
</tbody>
</table>

\(^\text{a}\)Missing value is included.

During follow-up period, two patients treated with cetuximab (epidermal growth factor receptor monoclonal antibody) for their metachronous metastases. The actuarial 5-year RFS and 5-year CSS were 62.0% and 76.0%, respectively.

https://doi.org/10.12701/yujm.2019.00157
Table 2. Univariate analysis of prognostic factors in entire group with residual rectal cancer patients after preoperative CRT

<table>
<thead>
<tr>
<th>Clinicopathological characteristic</th>
<th>n (%)</th>
<th>Recurrence free survival</th>
<th>Cancer-specific survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>p-value&lt;sup&gt;a&lt;/sup&gt;</td>
<td>HR</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>70 (47.9)</td>
<td>0.676</td>
<td>0.897</td>
</tr>
<tr>
<td>≥60</td>
<td>76 (52.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>102 (69.9)</td>
<td>0.853</td>
<td>1.053</td>
</tr>
<tr>
<td>Female</td>
<td>44 (30.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment CEA&lt;sup&gt;b&lt;/sup&gt; (ng/mL)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;5</td>
<td>91 (66.9)</td>
<td>0.580</td>
<td>1.168</td>
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<tr>
<td>≥5</td>
<td>45 (33.1)</td>
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<tr>
<td>Tumor distance from anal verge (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>112 (76.7)</td>
<td>0.609</td>
<td>1.170</td>
</tr>
<tr>
<td>≥5</td>
<td>34 (23.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual tumor size (cm)</td>
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<td></td>
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<tr>
<td>&lt;6.0</td>
<td>72 (49.3)</td>
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<td>1.830</td>
</tr>
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<td>≥6.0</td>
<td>74 (50.7)</td>
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<td></td>
</tr>
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<td>T1</td>
<td>4 (2.7)</td>
<td>&lt;0.001</td>
<td>0.705</td>
</tr>
<tr>
<td>T2</td>
<td>33 (22.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>100 (68.5)</td>
<td>2.045</td>
<td>0.281-14.870</td>
</tr>
<tr>
<td>T4</td>
<td>9 (6.2)</td>
<td>14.972</td>
<td>1.843-121.603</td>
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<td>ypN category</td>
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<td></td>
<td></td>
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<tr>
<td>N0</td>
<td>99 (67.8)</td>
<td>&lt;0.001</td>
<td>1.298</td>
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<td>N1</td>
<td>27 (18.5)</td>
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<td>N2</td>
<td>20 (13.7)</td>
<td>3.543</td>
<td>1.918-6.546</td>
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<td>Pathologic TNM stage</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>31 (21.2)</td>
<td>0.005</td>
<td>2.327</td>
</tr>
<tr>
<td>II</td>
<td>68 (46.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>47 (32.2)</td>
<td>3.833</td>
<td>1.575-9.326</td>
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<tr>
<td>Downstage</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>92 (63.0)</td>
<td>0.024</td>
<td>0.558</td>
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<tr>
<td>No</td>
<td>54 (37.0)</td>
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</tr>
<tr>
<td>Tumor regression grade</td>
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<td></td>
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<td>0</td>
<td>4 (2.7)</td>
<td>0.014</td>
<td>0.308</td>
</tr>
<tr>
<td>1</td>
<td>21 (14.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>55 (37.7)</td>
<td>0.232</td>
<td>0.069-0.774</td>
</tr>
<tr>
<td>3</td>
<td>66 (45.2)</td>
<td>0.173</td>
<td>0.052-0.582</td>
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<tr>
<td>Lymphatic invasion</td>
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<td></td>
<td></td>
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<td>112 (76.7)</td>
<td>0.004</td>
<td>2.161</td>
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<tr>
<td>Yes</td>
<td>34 (23.3)</td>
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<tr>
<td>Perineural invasion</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>No</td>
<td>112 (76.6)</td>
<td>&lt;0.001</td>
<td>2.811</td>
</tr>
<tr>
<td>Yes</td>
<td>34 (23.3)</td>
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<td></td>
<td></td>
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<tr>
<td>No</td>
<td>141 (96.6)</td>
<td>&lt;0.001</td>
<td>40.488</td>
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<tr>
<td>Yes</td>
<td>5 (3.4)</td>
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<tr>
<td>Circumferential resection margin (mm)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Positive (≤1)</td>
<td>30 (20.5)</td>
<td>&lt;0.001</td>
<td>3.563</td>
</tr>
<tr>
<td>Negative (&gt;1)</td>
<td>116 (79.5)</td>
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</tr>
<tr>
<td>KRAS mutation&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>76 (72.4)</td>
<td>0.107</td>
<td>1.662</td>
</tr>
<tr>
<td>Positive</td>
<td>29 (27.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRAF mutation&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>101 (96.2)</td>
<td>0.435</td>
<td>1.747</td>
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<tr>
<td>Positive</td>
<td>4 (3.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CEA, carcinoembryonic antigen; CRT, chemoradiotherapy; HR, hazard ratio; CI, confidence interval; TNM, tumor, node, metastasis.

<sup>a</sup><sup>p</sup>-values were calculated using log-rank test. <sup>b</sup>Missing value is included.
metastasis, for RFS, CRM positivity (HR=2.841; p=0.007); for CSS, larger residual tumor size (HR=3.589; p=0.028) and CRM (HR=4.257; p=0.011) were independent worse prognostic factors, respectively. Whereas, in patients with LNM, for RFS, CRM positivity (HR=3.011; p=0.005); for CSS, presence of perineural invasion (HR=2.973; p=0.027) and CRM positivity (HR=3.053; p=0.018) were independent negative prognostic factors, respectively.

Discussion

Accurate pathological examination of surgical specimens resected from rectal cancer patients treated with preoperative CRT is crucial in determining prognostic factors, such as pCR, that may affect patient outcome and subsequent therapeutic management. However, robust pathological factors of residual disease associated with patient outcome have yet to be fully elucidated. Therefore, analyzing known histoprognostic factors in patients with residual disease allows correlations with patient outcome to be evaluated. In our study, we investigated associations between histoprognostic factors and survival in patients with residual rectal cancer after surgical resection, who had received preoperative CRT. Our results revealed that residual tumor size, ypT category, ypN category, ypTNM stage, downstage, TRG, lymphatic invasion, perineural invasion, venous invasion, and CRM were significantly associated with RFS and/or CSS in patients with residual rectal cancer after preoperative CRT. Of these factors, CRM positivity was identified as an independent prognostic factor associated with poorer outcome with respect to RFS and CSS. Furthermore, CRM positivity was an independent predictor of reduced RFS and CSS, irrespective of subgrouping according to downstage or LNM. These findings are in strong accordance with the results of previous studies of rectal cancer patients treated with preoperative CRT, irrespective of pCR [2,10,11,21]. In these studies, Quah et al. [2] reported that "pathologic stage (ypTN stage)” was the most accurate predictor for DFS, while Gosens et al. [10] demonstrated that CRM positivity, lymph node status, and ypTNM stage were significantly associated with both local recurrence and overall survival on univariate analysis. In the latter study, CRM positivity was an independent predictor of local recurrence, but not of overall survival. In contrast, we determined that CRM positivity was an independent prognostic factor of both RFS and CSS, along with the ypTNM stage. Interestingly, a new staging system based on the combination of ypN stage and CRM status proposed by Gosens et al. was found to be a better prognostic tool than classic ypTNM staging in rectal cancer [11].

There is increasing evidence to suggest that CRM in rectal cancer is an important predictor of local recurrence, development of distant metastasis, and patient outcome [10,21-25]. While many studies have used the standard definition of ≤1 mm as a CRM positivity [22,26], others have reported that tumor cells within 2 mm from the margin were associated with an unfavorable prognosis [27,28]. In this regard, by comparing cutoff values in rectal cancer patients who had received preoperative CRT,
Non-down stage group

Recurrence free survival

CRM negative

CRM positive

Follow-up time (mon)

p<0.001

Down stage group

Recurrence free survival

CRM negative

CRM positive

Follow-up time (mon)

p=0.002

pN0 category group

Recurrence free survival

CRM negative

CRM positive

Follow-up time (mon)

p<0.001

Cancer specific survival

CRM negative

CRM positive

Follow-up time (mon)

p<0.001

Non-down stage group

Cancer specific survival

CRM negative

CRM positive

Follow-up time (mon)

p<0.001

p=0.002

p=0.001

CRM negative

CRM positive

Follow-up time (mon)

p<0.001

CRM negative

CRM positive

Follow-up time (mon)

p=0.001

Recurrence free survival

CRM negative

CRM positive

Follow-up time (mon)

p<0.001

Recurrence free survival

CRM negative

CRM positive

Follow-up time (mon)

p<0.001

Recurrence free survival

CRM negative

CRM positive

Follow-up time (mon)

p<0.001

Recurrence free survival

CRM negative

CRM positive

Follow-up time (mon)

p<0.001

Recurrence free survival

CRM negative

CRM positive

Follow-up time (mon)

p<0.001

Recurrence free survival

CRM negative

CRM positive

Follow-up time (mon)

p<0.001
Hwang et al. [19] demonstrated that 1 mm was an appropriate measurement for determining CRM positivity. Furthermore, Park et al. [18] demonstrated that a 1 mm cutoff value could be used to discern CRM positivity used in the prognosis of patient outcome, in rectal cancer patients with and without preoperative CRT. In our study, CRM positivity was defined as ≤1 mm and was observed in 20.5%. Because our cohort was constructed solely from patients with residual disease, the percentage of CRM positivity was relatively high, although within the reported range of 4-21% [2,10,18,19,29]. Our data suggest that CRM status may be as powerful as ypTNM staging as a prognostic indicator for patient outcome in patients with residual rectal cancer after preoperative CRT. Although CRM status has known prognostic value, we noted that CRM data were occasionally absent from pathology reports and they could not be assessed from paraffin blocks owing to a lack of embedding all clinically relevant areas. In addition, owing to the fibrotic response to CRT, tumor depth could not be accurately determined on gross examination of the surgical specimen of some patients. Furthermore, it has also been reported that vital tumor cells can be found scattered throughout the whole fibrotic area, resulting in CRM positivity [10]. Thus, we recommend that in the case of preoperative CRT rectal cancers, CRM assessment should be considered for all tissue surrounding the entire tumor CRM, and be documented within the pathology report. In addition, the pathologists should not hesitate to determine CRM positivity and the clinicians should not be afraid to decide accurate treatment strategies.

Interestingly in our study, TRG is associated with patient outcome but is not an independent prognostic factor in patients with residual rectal cancer after CRT. In addition, we observed only moderate reproducibility of TRG scoring between two pathologists, which was complicated by evaluating TRG solely in patients with residual tumor, also observed in a previous study [10]. This discrepancy highlights that using TRG to predict patient outcome in residual rectal cancer presents with some difficulty in practice.

We acknowledge that the data presented: (1) is a retrospective study from a single institution with a relatively homogeneous population; and (2) may have too few numbers in each patient subgroup to perform robust statistical analyses. Therefore, subsequent large-scale studies are needed to validate our results. Despite these limitations, this study is a comprehensive evaluation of clinicopathological prognostic factors associated with residual rectal cancer after preoperative CRT in an East Asian population.

In conclusion, CRM positivity was independent prognostic indicator that predicted for poorer patient outcome in relation to both RFS and CSS irrespective of ypTNM stage, in patients with residual rectal cancer who received preoperative CRT. Additionally, in these patients, CRM positivity retained significance as an independent prognostic indicator of poor outcome for RFS and CSS, irrespective of downstage or LNM. Finally, these results have implications for the stratification of...
Table 3. Univariate analysis of prognostic factors in subgrouping patients according to downstage and LNM after preoperative CRT

<table>
<thead>
<tr>
<th>Clinicopathological characteristic</th>
<th>Non-downstage group (n (%)</th>
<th>Downstage group (n (%</th>
<th>non LNM group (n (%</th>
<th>LNM group (n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value^a</td>
<td>p-value^a</td>
<td>p-value^a</td>
<td>p-value^a</td>
</tr>
<tr>
<td>Pretreatment CEA (ng/mL)^b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>30 (58.8)</td>
<td>0.659</td>
<td>26 (78.8)</td>
<td>0.282</td>
</tr>
<tr>
<td>≥5</td>
<td>21 (41.2)</td>
<td>0.664</td>
<td>20 (21.2)</td>
<td>0.095</td>
</tr>
<tr>
<td>Tumor distance from anal verge (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>40 (74.1)</td>
<td>0.414</td>
<td>32 (78.8)</td>
<td>0.736</td>
</tr>
<tr>
<td>≥5</td>
<td>14 (25.9)</td>
<td>0.222</td>
<td>10 (21.2)</td>
<td>0.12</td>
</tr>
<tr>
<td>Residual tumor size (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;6.0</td>
<td>23 (42.6)</td>
<td>0.451</td>
<td>19 (42.6)</td>
<td>0.024</td>
</tr>
<tr>
<td>≥6.0</td>
<td>31 (57.4)</td>
<td>0.354</td>
<td>23 (47.5)</td>
<td>0.008</td>
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<td>ypT category</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
<td>0 (0)</td>
<td>0.004</td>
</tr>
<tr>
<td>T2</td>
<td>6 (11.1)</td>
<td>&lt;0.001</td>
<td>4 (4.0)</td>
<td>0.004</td>
</tr>
<tr>
<td>T3</td>
<td>41 (75.9)</td>
<td>0.003</td>
<td>27 (27.3)</td>
<td>0.004</td>
</tr>
<tr>
<td>T4</td>
<td>7 (10.0)</td>
<td>&lt;0.001</td>
<td>2 (2.0)</td>
<td>0.004</td>
</tr>
<tr>
<td>Tumor regression grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2 (3.7)</td>
<td>&lt;0.001</td>
<td>2 (2.0)</td>
<td>0.004</td>
</tr>
<tr>
<td>1</td>
<td>8 (14.8)</td>
<td>&lt;0.001</td>
<td>13 (13.1)</td>
<td>0.004</td>
</tr>
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<td>2</td>
<td>22 (40.7)</td>
<td>0.845</td>
<td>26 (36.4)</td>
<td>0.004</td>
</tr>
<tr>
<td>3</td>
<td>22 (40.7)</td>
<td>0.397</td>
<td>48 (48.5)</td>
<td>0.004</td>
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<td>Lymphatic invasion</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>25 (46.3)</td>
<td>0.041</td>
<td>87 (94.6)</td>
<td>0.924</td>
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<td>29 (53.7)</td>
<td>0.035</td>
<td>5 (5.4)</td>
<td>0.287</td>
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<td>Perineural invasion</td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>80 (87.0)</td>
<td>0.286</td>
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<td>12 (13.0)</td>
<td>0.105</td>
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<td>Venous invasion</td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>49 (90.7)</td>
<td>&lt;0.001</td>
<td>92 (100)</td>
<td>0.483</td>
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<td>5 (9.3)</td>
<td>&lt;0.001</td>
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<tr>
<td>Circumferential resection margin (mm)</td>
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<td></td>
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<tr>
<td>≤1</td>
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<td>&lt;0.001</td>
<td>72 (78.8)</td>
<td>0.002</td>
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<td>&gt;1</td>
<td>10 (18.5)</td>
<td>&lt;0.001</td>
<td>20 (21.2)</td>
<td>0.002</td>
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<tr>
<td>KRAS mutation^c</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>29 (69.0)</td>
<td>0.382</td>
<td>47 (74.6)</td>
<td>0.085</td>
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<tr>
<td>Positive</td>
<td>13 (31.0)</td>
<td>0.631</td>
<td>16 (25.4)</td>
<td>0.28</td>
</tr>
<tr>
<td>BRAF mutation^d</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Negative</td>
<td>39 (92.9)</td>
<td>0.223</td>
<td>62 (67.4)</td>
<td>0.483</td>
</tr>
<tr>
<td>Positive</td>
<td>3 (7.1)</td>
<td>0.204</td>
<td>1 (1.1)</td>
<td>0.672</td>
</tr>
</tbody>
</table>

LNM, lymph node metastasis; CEA, carcinoembryonic antigen; CRT, chemoradiotherapy; RFS, recurrence free survival; CSS, cancer-specific survival, NE, not evaluated.

^a-p-values were calculated using log-rank test. ^bMissing value is included.

Table 4. Multivariate analysis to determine the independent prognostic factors in entire group with residual rectal cancer patients after preoperative CRT

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Recurrence free survival (multivariate analysis)</th>
<th>Cancer-specific survival (multivariate analysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>p-value</td>
<td>HR</td>
</tr>
<tr>
<td>Pathologic TNM stage</td>
<td>III vs. I&amp;II</td>
<td>0.105</td>
<td>1.744</td>
</tr>
<tr>
<td>Residual tumor size (cm)</td>
<td>≥6 vs. &lt;6</td>
<td>0.049</td>
<td>1.753</td>
</tr>
<tr>
<td>Venous invasion</td>
<td>Present vs. absent</td>
<td>&lt;0.001</td>
<td>20.425</td>
</tr>
<tr>
<td>Lymphatic invasion</td>
<td>Present vs. absent</td>
<td>0.889</td>
<td>1.050</td>
</tr>
<tr>
<td>Perineural invasion</td>
<td>Present vs. absent</td>
<td>0.117</td>
<td>1.667</td>
</tr>
<tr>
<td>CRM</td>
<td>Positive vs. negative</td>
<td>&lt;0.001</td>
<td>3.613</td>
</tr>
<tr>
<td>Tumor regression grade</td>
<td>0-8 vs. 2&amp;3</td>
<td>0.425</td>
<td>1.311</td>
</tr>
</tbody>
</table>

CRT, chemoradiotherapy; HR, hazard ratio; CI, confidence interval; TNM, tumor, node, metastasis, vs., versus; CRM, circumferential resection margin.
patients at a higher risk of disease progression, and may help clinicians implement more effective treatment strategies in patients presenting with residual rectal cancer after CRT.

Acknowledgements

This study was partly supported by a grant in-aid from Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (grant number: NRF-2015R1C1A1A02037597).

Conflicts of interest

No potential conflicts of interest relevant to this article was reported.

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Factors affecting complications after treatment of epidermal cyst

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Background: Epidermal cysts are the most common benign epithelial tumors in humans. The curative treatment of epidermal cyst is surgical excision. However, only few studies have investigated the cause and mechanism of postoperative complications of epidermal cysts. Therefore, this study aimed to evaluate the factors affecting complications of epidermal cyst after surgical treatment.

Methods: Patients with histologically diagnosed epidermal cysts were selected from among 98 consecutive patients with excised benign cystic tumors from March 2014 to August 2017. Sex, age, size, mobility, site of occurrence, history of infection, history of incision and drainage, complications, history of reoperation, and method of overcoming complications was obtained by analyzing medical records retrospectively.

Results: Five of the 98 patients had wound dehiscence due to surgical infection. Three of them underwent wound healing with conservative treatment without a second operation. The other two patients underwent a second operation and showed signs of preoperative infection. None of the factors showed statistical significance in relation to the occurrence of complications.

Conclusion: Postoperative complications occurred when the excision of the epidermal cyst was performed at preoperative infection sites or at sites with high tension, so attention should be paid to postoperative care.

Keywords: Complications; Epidermal cyst; Surgical wound infection

Introduction

Epidermal cysts are the most common benign epithelial tumors in humans [1]. Epidermal cysts can occur anywhere in the body, especially on the face (Fig. 1). Epidermal cysts mostly manifest as a mass, but some cysts may appear as granulation tissue with foreign-body granulomatous reaction and chronic inflammation when infection and cyst wall rupture occur [2].

The curative treatment of epidermal cyst is surgical excision [3]. Several treatment methods, including minimal incision surgery, have been reported [4]. However, surgical treatment results in scarring of various lengths. Postoperative complications include infection, bleeding, scarring, and cyst recurrence [2]. In infected epidermal cysts, delayed excision is performed after incision and drainage is performed after infection control (Fig. 2).

However, only few studies have investigated the cause and mechanism of postoperative complications [5]. Therefore, this study aimed to evaluate the factors affecting the post-surgical complications of epidermal cyst treatment.
Materials and methods

The study was performed in accordance with the principles of the Declaration of Helsinki. Written informed consents were obtained. The present study was approved by the Institutional Review Committee of Yeungnam University hospital (YUMC 2018-06-002-001). The patient provided written informed consent for the publication and the use of his images.

Patients with histologically diagnosed epidermal cysts were selected from among 98 consecutive patients with excised benign cystic tumors from March 2014 to August 2017. Sex, age, size, mobility, site of occurrence, history of infection, history of incision and drainage, complications, history of reoperation, and method of overcoming complications were obtained by analyzing medical records retrospectively. Those with erythema or swelling were considered as having a history of infection. Scarring after surgery was not considered a complication.

If signs of infection, such as erythema or swelling, were observed before the operation, incision and drainage were performed, and the excision was performed after the infection was controlled. After incision and drainage, disinfection treatments such as irrigation were performed daily, and infection was considered to be controlled if discharge or erythema was not observed.

Primary closure was performed after excision in all the patients. Twenty-six patients with preoperative signs of infection were treated with daily irrigation, and excision was performed within 2 weeks.

1. Surgical procedures

The skin overlying the epidermal cyst was cleaned with chlorohexidine and physiological saline. Surgery was performed under local anesthetic infiltration of 2% lidocaine with 1:100,000 epinephrine. The elliptical-shaped incision line was designed to run parallel to the relaxed skin tension line, not crossing over the area of protuberance, and to include the opening of the epidermal cyst. En bloc resection without separating skin and the cyst in the dermal layer was made using a Metzenbaum scissor and electrocauterization. When the cyst wall ruptured during surgery, the suspicious portion of the cyst wall was resected after sufficient irrigation. At the end of the procedure, the wound was inspected to ensure that all of the cyst wall was removed. The face and anterior side of the neck were closed using a nylon 6-0 or 7-0 suture, and the trunk and extremity were closed using a nylon 4-0 or 5-0 suture.

2. Statistical analysis

The statistical analysis was performed using IBM SPSS version 24.0 (IBM Co., Armonk, NY, USA). An independent sample t test was used to evaluate the correlation between postoperative complications and age. Correction between the postoperative complications and the size of epidermal cyst were analyzed using the MannWhitney test. The chi-square test was used to evaluate the correlation between the postoperative complications and other factors.

Results

Of the 98 patients included in the study (Table 1), 74 were men and 24 were women. The mean age was 42.71 years (range, 3-91 years). The mean size was 12 mm (range, 3-89 mm). Among the 98 cases, 36 had mobility. The distribution of the sites was 48 on the face, 20 on the trunk, 11 on the ear, eight on the neck, eight on the extremity, and three on the scalp. In 26 patients, preoperative signs of infection were observed, and incision and drainage were performed. In 14 patients, cyst wall rupture was observed on
histopathological examination. All the patients were diagnosed as having an epidermal cyst based on histopathological examination.

No significant differences were found between the postoperative complications and age (p=0.322). No statistical significance was observed between the postoperative complications and epidermal cyst size (p=0.267). No significant difference was found between postoperative complications and sex (p=0.093), between postoperative complications and preoperative infection (p=0.606), between postoperative complications and cyst wall rupture (p=0.301), between postoperative complications and site of occurrence (p=0.517), and between postoperative complications and mobility (p=0.924) (Table 2).

Surgical site infection was the only complication except scarring. Five patients had wound dehiscence due to surgical site infection (Fig. 3). Five wound sites showed signs of postoperative infection were the back in two, neck in two, and chin in one (Table 3). Three

![Fig. 3. Wound dehiscence due to postoperative surgical infection. (A) Before stitch out. (B) After stitch out.](https://doi.org/10.12701/yujm.2019.00164)
of the sites underwent wound healing with conservative treatment without a second operation. They showed no signs of infection before surgery, but the cyst walls were ruptured as shown in the biopsy (Fig. 4). The other two patients underwent a second operation and had signs of preoperative infection. The surgical sites were the posterior neck and back, respectively.

**Discussion**

In this study, we investigated the association of various factors and complications that may occur after excision of epidermal cysts. Complications, except for scarring, occurred after excision of the epidermal cyst, which caused wound dehiscence due to infection. Complications occurred in cases with preoperative infection or excised cyst wall rupture. The sites of complications were the back, posterior neck, and chin areas, where tension is relatively high in the body.

Epidermal cysts are smooth, dome-shaped, freely movable, somewhat fluctuant subcutaneous swelling, which are sometimes attached to the skin by a central pore. The cysts are covered with a stratified squamous epithelium that resembles the epidermis or follicular infundibulum.

The first-line treatment of epidermal cyst is simple excision [3]. It is easy to excise if no infection signs are evident. However, in cases with epidermal cyst infection, excision is often difficult because of tissue inflammation or cyst wall rupture. If the cyst wall is not completely removed, the epidermal cyst would recur. Therefore, when an epidermal cyst is infected, surgeons should consider partially incising the cyst and draining it first, and then excising it completely 1–2 weeks later.

Recently, many studies have shown that epidermal cysts are associated with postoperative scar formation. Minimal incision or laser is used to remove the epidermal cyst with minimal injury to the skin [3]. This helps minimize scar formation after excision of the epidermal cyst. However, only few studies have been conducted on complications after epidermal cyst excision.

In preoperative infection of the epidermal cyst, the cyst wall ruptures and pus or blood in the cyst comes out and infection often follows. The sites of complication after excision of the epidermal cyst are the back, posterior neck, and chin, which have relatively high tension. When an epidermal cyst occurs at the site where a lot of tension is applied, the cyst wall is well ruptured.
owing to an external mechanical force. When the epidermal cyst is excised, the cyst wall could not be easily completely removed if it is ruptured. If the wound is closed while the cyst wall remains, infection may occur and the risk of subsequent recurrence increases.

The limitation of this study is that no statistical significance was attained. None of the factors showed statistical significance in relation to the occurrence of complications. We conclude that the lack of statistical significance was because of the inadequate number of cases in the group with complications. Statistical significance must be confirmed by continuing additional studies.

Postoperative complications occurred when the excision of the epidermal cyst was performed at preoperative infection sites or at sites with high tension. When excision of the epidermal cyst is performed at preoperative infection sites or at sites with high tension, attention should be paid to postoperative care.

Acknowledgements

This work was supported by a grant from the Chunma medical research foundation, Korea, 2017.

Conflicts of interest

No potential conflicts of interest relevant to this article was reported.

References


Patient consent

Patients provided written consent for the use of their images.

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Association between cadmium exposure and hearing impairment: a population-based study in Korean adults

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**Background:** The present study aimed to evaluate the clinical association between cadmium exposure and hearing impairment among the Korean population.

**Methods:** This retrospective cross-sectional study used the data obtained from the Korean National Health and Nutrition Examination Survey were used for our study. Finally, 3,228 participants were included in our study, which were then divided into quartiles based on their blood cadmium levels: first quartile (1Q), second quartile (2Q), third quartile (3Q), and fourth quartile (4Q) groups. The hearing thresholds were measured using an automatic audiometer at 0.5, 1, 2, 3, 4, and 6 kHz. Hearing loss (HL) was defined as >25 dB average hearing threshold (AHT).

**Results:** All the groups had 807 participants each. The area under the receiver operating characteristic curves of cadmium level for HL were 0.634 (95% confidence interval [CI], 0.621–0.646). The participants in the 4Q group had higher Low/Mid-Freq, High-Freq, and AHT values than those in the other groups in the multivariate analysis after adjusting for confounding factors. The logistic regression showed that the OR for HL per 1 µg/L increase in cadmium was 1.25 (95% CI, 1.09–1.44; p=0.002) on the multivariate analysis. Moreover, the multivariate logistic regression analyses revealed that the participants in the 4Q group exhibited a 1.59-, 1.38-, and 1.41-fold higher odds for HL than those in the 1Q, 2Q, and 3Q groups, respectively.

**Conclusion:** High cadmium level quartile was associated with increased hearing thresholds and HL among the Korean adult population.

**Keywords:** Cadmium; Hearing loss; Heavy metals

**Introduction**

Hearing loss (HL) is one of the health problems with the highest prevalence. The World Health Organization (WHO) showed that 360 million individuals are suffering from HL, with its health-care cost amounting to approximately $750 billion globally [1]. HL is associated with decreased quality of life and social communication. Therefore, early detection of HL and identification of its risk factors are important health issues worldwide. Environmental or industrial exposure to various heavy metals is an essential cause of hearing impairment among the general population.

Cadmium is a toxic, nonessential transition metal, and its accumulation in the body is associated with cancer or neurotoxicity development [2]. Many studies have shown that cadmium causes ototoxicity through various mechanisms. An animal study revealed that cadmium-exposed rat developed abnormalities in the auditory brainstem response waves, and changes in the ionic composition of the inner ear perilymph were observed [3]. Kim et al. showed that cadmium causes reactive oxygen species generation in the cochlear cells, which results in increased hearing thresholds [4].
In contrast to many in vitro or in vivo studies, population-based studies regarding the association between cadmium exposure and hearing impairment are sparse. Choi et al. investigated 3,698 American adults who were randomly assigned to the Audiometry Examination Component of the National Health and Nutrition Examination Survey (NHANES), and their results showed a positive association between cadmium exposure and hearing impairment [5]. Shiue used a data set from a different period in the same survey, but they did not find an association between cadmium exposure and HL [6]. Except for the previous two studies, few studies on the association between cadmium exposure and HL among the adult populations are available. Therefore, further research is needed to identify the relationship between the two. The present study aimed to evaluate the clinical association between cadmium exposure and hearing impairment among the Korean population.

**Materials and methods**

**1. Study population**

This retrospective cross-sectional study used the data obtained from the 2010–2012 Korean National Health and Nutrition Examination Survey (KNHANES) were used for our study. The KNHANES is a nationwide, multi-stage, stratified survey of a representative sample of the South Korean population conducted by the Korea Centers for Disease Control and Prevention. A total of 10,787 participants aged between 40 and 70 years. Among them, 1,351 and 6,209 participants whose data on hearing thresholds and cadmium levels, respectively, were unavailable were excluded from the study. Finally, 3,228 participants were included in our study, which were then divided into quartiles based on their blood cadmium levels: first quartile (1Q), second quartile (2Q), third quartile (3Q), and fourth quartile (4Q) groups. Kyungpook National University Hospital Institutional Review Board approval was obtained for our study (KNUH 2018-11-009).

**2. Study variables**

The following clinical and laboratory data were collected from the participants during the health examination: cadmium level, lead level, age, sex, presence of diabetes mellitus (DM), presence of hypertension (HTN), smoking habit, alcohol intake, explosive and occupational noise exposure, and hearing thresholds.

DM was defined as a self-reported history of a DM diagnosis, use of hypoglycemic drugs, ≥126 mg/dL fasting glucose level, or ≥6.5% HbA1c. HTN was defined as ≥140 mmHg systolic blood pressure, ≥90 mmHg diastolic blood pressure, self-reported history of HTN, or use of anti-hypertensive drugs. The participants were classified based on their smoking status as either current smokers (consumed ≥100 cigarettes in their lifetime and ceased smoking <1 year before the survey), ex-smokers (consumed ≥100 cigarettes in their lifetime and ceased smoking ≥1 year before the survey), or non-smokers (consumed <100 cigarettes in their lifetime). Alcohol intake was defined using the Korean version of standard drinking, which is based on the WHO classification system [7]. We classified the alcohol intake into the following categories: abstinence (no alcohol consumption during the 12 months prior to the evaluation), moderate consumption (women, 0.1–19.99 g pure alcohol/day; men, 0.1–39.99 g pure alcohol/day), and heavy consumption (women, ≥20 g pure alcohol/day; men, ≥40 g pure alcohol/day).

Histories of exposure to explosive and occupational noise were classified as positive or negative, as previously described [8]. An explosive noise was defined as a sudden loud noise, such as an explosion or gunshot. Exposure to occupational noise was positive if participants had worked in a location with loud machinery for ≥3 months. Additionally, exposure to loud noise was positive if participants needed to raise their voice to have a conversation. The hearing thresholds were measured using an automatic audiometer at 0.5, 1, 2, 3, 4, and 6 kHz. For both ears of each subject, the threshold values at 0.5, 1, and 2 kHz were averaged to obtain the low- or middle-frequency pure-tone average (Low/Mid-Freq), and the values at 3, 4, and 6 kHz were averaged to obtain the high-frequency pure-tone average (High-Freq). The average hearing threshold (AHT) was calculated as the pure-tone average of the thresholds at 0.5, 1, 2, and 3 kHz. HL was defined as >25 dB AHT.

The cadmium levels in the blood were measured using graphite furnace atomic absorption spectrometry (Analyst 600; PerkinElmer, Turku, Finland) and presented as µg/L. All blood cadmium analyses were performed by the Neodin Medical Institute, a laboratory certified by the Ministry of Health and Welfare of Korea. The internal quality assurance and control was conducted 4 times every month using four commercial reference materials (Lyphochek Whole Blood Metals Control; Bio-Rad Laboratories, Hercules, CA, USA) with different concentrations, and most of the results were within the allowable range. The coefficients of the blood cadmium variations were within 0.95–4.82% in the reference samples. The external quality assurance and control was performed 4 times every year; the institute passed both the German External Quality Assessment Scheme and Quality Assurance Program operated by Friedrich-Alexander University and Korea Occupational Safety and Health Agency, respectively [9].

**Statistical analyses**

The data were analyzed using the IBM SPSS version 19 (IBM Co.,
Armonk, NY, USA). The categorical and continuous variables were expressed as counts (%) and means±standard error (SE) or standard deviation, respectively. Pearson’s $\chi^2$ or Fisher’s exact test was used to analyze the categorical variables. Meanwhile, the means of the continuous variables were compared using one-way analysis of covariance, followed by a post-hoc Tukey’s comparison.

We calculated the sensitivity, specificity, cutoff values, and probability of the area under the receiver operating characteristic curve (AUROC) to predict the HL using cadmium level. The strength of the relationship between the continuous variables was assessed using correlation coefficients. A linear regression analysis was performed to examine the independent predictors of the hearing thresholds. Logistic regression analyses were used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs), which were then utilized to determine the correlation between cadmium level and HL. Model 1 were adjusted for age, sex, presence of DM and HTN, smoking habit, alcohol intake, and explosive or occupational noise exposure. Model 2 were adjusted for variables for Model 1 and lead level. The multivariate analyses were performed using analysis of covariance and linear or logistic regression analysis. $p$-values <0.05 were considered statistically significant.

## Results

### 1. Participants’ clinical characteristics

All the groups had 807 participants each (Table 1). The mean cadmium values (interval) in the 1Q, 2Q, 3Q, and 4Q groups were 0.67 (0.10–0.87), 1.05 (0.86–1.21), 1.41 (1.22–1.65), and 2.25 (1.66–6.42) µg/L, respectively. The participants in the 1Q group had lower mean age than those of the other groups. Meanwhile, the 4Q group displayed higher proportions of participants who had HTN, current smoking habits, and heavy alcohol intake than the other groups. Furthermore, the 1Q group had higher proportion of participants who were exposure to explosive noise than the other groups. The mean lead values in the 1Q, 2Q, 3Q, and 4Q groups were 2.41±1.23, 2.54±1.10, 2.69±1.43, and 2.81±1.41 µg/dL, respectively ($p<0.001$).

### 2. Association between cadmium level and hearing impairment

The AUROCs of cadmium level for HL were 0.634 (95% CI, 0.621–0.646; Fig. 1). The sensitivity and specificity for predicting HL were 64.8% (95% CI, 61.4–68.0) and 55.5% (95% CI, 54.1–56.9), respectively ($p<0.001$).

In the univariate analysis, participants in the 4Q group showed higher Low/Mid-Freq, High-Freq, and AHT values than those in 1Q group (Fig. 2). Similarly, the participants in the 4Q group had higher Low/Mid-Freq, High-Freq, and AHT values than those in the other groups in the multivariate analysis after adjusting for confounding factors. The numbers of participants with HL in the 1Q, 2Q, 3Q, and 4Q groups were 153 (19.0%), 169 (20.9%), 163 (20.2%), and 202 (25.1%), respectively ($p=0.018$). The logistic regression showed that the OR for HL per 1 µg/L increase in

### Table 1. Participants’ clinical characteristics based on the cadmium level quartiles

<table>
<thead>
<tr>
<th>Variable</th>
<th>1Q (n=807)</th>
<th>2Q (n=807)</th>
<th>3Q (n=807)</th>
<th>4Q (n=807)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>52.7±9.0</td>
<td>54.2±8.7$^a$</td>
<td>55.0±8.5$^a$</td>
<td>54.8±8.2$^a$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex (men, %)</td>
<td>549 (68.0)</td>
<td>431 (53.4)</td>
<td>338 (41.9)</td>
<td>292 (36.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>123 (15.2)</td>
<td>109 (13.5)</td>
<td>103 (12.8)</td>
<td>104 (12.9)</td>
<td>0.445</td>
</tr>
<tr>
<td>Hypertension</td>
<td>282 (35.0)</td>
<td>303 (37.5)</td>
<td>316 (39.2)</td>
<td>344 (42.7)</td>
<td>0.014</td>
</tr>
<tr>
<td>Smoking habit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>400 (50.3)</td>
<td>427 (53.8)</td>
<td>466 (58.2)</td>
<td>465 (58.3)</td>
<td></td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>295 (37.1)</td>
<td>216 (27.2)</td>
<td>136 (17.0)</td>
<td>83 (10.4)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>101 (12.7)</td>
<td>150 (18.9)</td>
<td>199 (24.8)</td>
<td>250 (31.3)</td>
<td></td>
</tr>
<tr>
<td>Alcohol intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td>Abstinence</td>
<td>179 (22.6)</td>
<td>211 (26.7)</td>
<td>209 (26.2)</td>
<td>233 (29.2)</td>
<td></td>
</tr>
<tr>
<td>Moderate alcohol</td>
<td>580 (73.2)</td>
<td>540 (68.3)</td>
<td>542 (67.8)</td>
<td>508 (63.7)</td>
<td></td>
</tr>
<tr>
<td>Heavy alcohol</td>
<td>33 (4.2)</td>
<td>40 (5.1)</td>
<td>48 (6.0)</td>
<td>56 (7.0)</td>
<td></td>
</tr>
<tr>
<td>Exposure to occupational noise</td>
<td>28 (3.5)</td>
<td>42 (5.2)</td>
<td>36 (4.5)</td>
<td>48 (6.0)</td>
<td>0.087</td>
</tr>
<tr>
<td>Exposure to explosive noise</td>
<td>70 (8.7)</td>
<td>61 (7.6)</td>
<td>53 (6.6)</td>
<td>57 (7.1)</td>
<td>0.032</td>
</tr>
</tbody>
</table>

The data were expressed as number (%) and mean±standard deviations for categorical and continuous variables, respectively. 1Q, first quartile; 2Q, second quartile; 3Q, third quartile; 4Q, fourth quartile.

$p$-values were tested using one-way analysis of variance, followed by a post-hoc Tukey’s comparison for continuous variables and Pearson’s $\chi^2$ or Fisher’s exact tests for categorical variables.

$^a$ $p<0.05$ vs. 1Q.
cadmium was 1.17 (95% CI, 1.04–1.32; p<0.001), 1.27 (95% CI, 1.11–1.46; p=0.001), and 1.25 (95% CI, 1.09–1.44; p=0.002) on the univariate, model 1, and 2 analyses, respectively. Moreover, the model 2 logistic regression analyses revealed that the participants in the 4Q group exhibited a 1.59-, 1.38-, and 1.41-fold higher odds for HL than those in the 1Q, 2Q, and 3Q groups, respectively (Table 2).

The correlation coefficients of cadmium levels were 0.062, 0.016, and 0.054 for Low/Mid-Freq, High-Freq, and AHT, respectively (p<0.001 for Low/Mid-Freq, p=0.357 for High-Freq, and p=0.002 for AHT). The partial correlation coefficients of cadmium levels after adjusting for covariates of model 2 were 0.062, 0.085, and 0.072 for Low/Mid-Freq, High-Freq, and AHT, respectively (p<0.001 for all three thresholds). Additionally, the linear regression revealed that the unstandardized β±SE for cadmium level were 0.054±0.360, 0.083±0.363, and 0.074±0.367 on the univariate, model 1, and 2 analyses, respectively (Table 3). Positive associations between cadmium levels and AHT values were found.

**Fig. 1.** Receiver operating characteristic curve of cadmium level for the prediction of hearing loss.

**Fig. 2.** Hearing thresholds based on cadmium level quartiles. For the univariate analysis, the 1Q, 2Q, 3Q, and 4Q groups had mean Low/Mid-Freq values of 15.3±0.5, 16.5±0.5, 16.7±0.4, and 17.9±0.5, respectively; mean High-Freq values of 17.2±0.5, 18.3±0.5, 18.2±0.5, and 19.7±0.5, respectively. For the multivariate analysis, the 1Q, 2Q, 3Q, and 4Q groups had mean Low/Mid-Freq values of 15.1±0.5, 16.2±0.4, 16.6±0.4, and 18.0±0.4, respectively; mean High-Freq values of 28.0±0.7, 30.3±0.6, 30.7±0.6, and 33.7±0.6, respectively; mean AHT values of 16.6±0.5, 17.9±0.5, 18.3±0.5, and 20.1±0.5, respectively. The data are expressed as mean and standard error. The model 1 for hearing thresholds was adjusted for age, sex, presence of DM and hypertension, smoking habit, alcohol intake, occupational and explosive noise exposure. The model 2 for hearing thresholds was adjusted for age, sex, presence of DM and hypertension, smoking habit, alcohol intake, occupational and explosive noise exposure, and lead level. a)p<0.05 vs. 1Q, b)p<0.05 vs. 2Q, c)p<0.05 vs. 3Q. 1Q, first quartile; 2Q, second quartile; 3Q, third quartile; 4Q, fourth quartile; Low/Mid-Freq, low or middle frequency; High-Freq, high frequency; AHT, average hearing threshold; DM, diabetes mellitus.
Discussion

Our study showed that the 4Q group had the highest values for all the three hearing thresholds (Low/Mid-Freq, High-Freq, and AHT) among the four groups. The multivariate analyses showed that increased cadmium level was associated with increased odds for HL. Furthermore, the participants from the 4Q group had higher odds for HL than those in the other groups. The cadmium level as a continuous variable was positively correlated with AHT on both univariate and multivariate analyses. Moreover, the linear regression analyses also revealed similar trends.

Cadmium is a well-known environmental or industrial toxin, and an individual comes in contact with this substance through dietary intake, smoking, or polluted air inhalation [10]. Previous studies have shown the possible pathophysiology of cadmium-induced hearing impairment using in vitro and in vivo models, which suggested that cadmium induced damage to various cells, including hair cells, spiral ganglion neurons, and auditory neurons [2,4,11]. To the best of our knowledge, five studies on the association between cadmium level and hearing impairment among the general population were published. However, these studies involved inhomogeneous study population and reported inconsistent results.

Thatcher et al. first investigated the association between cadmium level and HL [12]. They evaluated the cadmium level in the hair and showed that it was associated with impaired auditory evoked potential. However, they did not assess the hearing thresholds and only enrolled children aged 5–16 years old. Shargorodsky et al. were the second to investigate the association between cadmium level and hearing impairment [13]. They

Table 2. Logistic regression analyses of hearing loss based on cadmium level quartiles

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p-value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Per 1 µg/L increase in cadmium</td>
<td>1.17 (1.04–1.32)</td>
<td>0.011</td>
<td>1.27 (1.11–1.46)</td>
</tr>
<tr>
<td>1Q vs. 2Q</td>
<td>1.13 (0.89–1.46)</td>
<td>0.319</td>
<td>1.15 (0.89–1.50)</td>
</tr>
<tr>
<td>1Q vs. 3Q</td>
<td>1.08 (0.85–1.38)</td>
<td>0.531</td>
<td>1.15 (0.88–1.51)</td>
</tr>
<tr>
<td>1Q vs. 4Q</td>
<td>1.43 (1.13–1.81)</td>
<td>0.003</td>
<td>1.65 (1.25–2.17)</td>
</tr>
<tr>
<td>2Q vs. 3Q</td>
<td>0.71 (0.96–1.22)</td>
<td>0.715</td>
<td>0.99 (0.76–1.29)</td>
</tr>
<tr>
<td>2Q vs. 4Q</td>
<td>1.26 (1.00–1.59)</td>
<td>0.049</td>
<td>1.40 (1.09–1.81)</td>
</tr>
<tr>
<td>3Q vs. 4Q</td>
<td>1.32 (1.05–1.67)</td>
<td>0.020</td>
<td>1.43 (1.11–1.83)</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; 1Q, first quartile; vs, versus; 2Q, second quartile; 3Q, third quartile; 4Q, fourth quartile.

Model 1 for hearing loss was adjusted for age, sex, presence of diabetes mellitus and hypertension, smoking habit, alcohol intake, and occupational and explosive noise exposure. Model 2 for hearing loss was adjusted for age, sex, presence of diabetes mellitus and hypertension, smoking habit, alcohol intake, occupational and explosive noise exposure, and lead level.

Table 3. Linear regression analyses of AHT based on the variables

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Univariate</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>0.054±0.360</td>
<td>0.002</td>
<td>0.083±0.363</td>
</tr>
<tr>
<td>Age</td>
<td>0.305±0.642</td>
<td>&lt;0.001</td>
<td>0.274±0.654</td>
</tr>
<tr>
<td>Sex</td>
<td>0.079±0.481</td>
<td>&lt;0.001</td>
<td>-0.113±0.683</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.102±0.700</td>
<td>&lt;0.001</td>
<td>0.037±0.677</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.162±0.487</td>
<td>&lt;0.001</td>
<td>0.092±0.482</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>0.048±0.294</td>
<td>0.007</td>
<td>-0.010±0.405</td>
</tr>
<tr>
<td>Alcohol intake</td>
<td>-0.052±0.457</td>
<td>0.003</td>
<td>-0.040±0.461</td>
</tr>
<tr>
<td>Exposure to occupational noise</td>
<td>0.134±0.216</td>
<td>&lt;0.001</td>
<td>0.188±0.580</td>
</tr>
<tr>
<td>Exposure to explosive noise</td>
<td>0.001±0.021</td>
<td>0.965</td>
<td>-0.236±0.586</td>
</tr>
</tbody>
</table>

AHT, average hearing threshold; US-β, unstandardized β; SE, standard error.

The dependent variable was the AHT levels, and the model 1 was adjusted for age, sex, presence of diabetes mellitus and hypertension, smoking habit, alcohol intake, and occupational and explosive noise exposure. Model 2 was adjusted for age, sex, presence of diabetes mellitus and hypertension, smoking habit, alcohol intake, occupational and explosive noise exposure, and lead level.

https://doi.org/10.12701/yujm.2019.00178
used representative data (NHANES) and enrolled adolescents aged 12–19 years old. Furthermore, they evaluated the cadmium level of the participants’ urinary samples. Their results showed that participants in the highest urinary cadmium level quartile had higher odds for low-frequency HL than those in the lowest quartile. Additionally, their finding revealed the absence of statistical significance between cadmium level and high-frequency HL. Two more recent studies were conducted which involved the adult population [5,6]. Choi et al. also used representative data (NHANES) and enrolled adults aged 20–60 years old to evaluate the relationship between blood cadmium level and hearing thresholds [5]. Their results revealed statistically significant HL only between the lowest and highest quintile groups. Shiue utilized the same data (2003–2004 NHANES) and enrolled adults aged ≥50 years old [6]. They evaluated the cadmium level through the participants’ urinary samples, whereas the degree of hearing impairment was determined using a questionnaire (good, little trouble, or poor). Their findings showed the absence of an association between cadmium level and hearing impairment. All the aforementioned studies revealed conflicting results on the association between the two variables, and half of them enrolled only children. Additionally, all studies were conducted among the US populations.

We used representative data from KNHANES and enrolled Korean adult population. We divided the hearing thresholds into three groups: Low/Mid-Freq, High-Freq, and AHT. Furthermore, we defined HL as a dependent variable and performed adjustments for potentially important confounding factors. Our results revealed that the participants in the highest cadmium level quartile had higher thresholds of or odds for HL than those in the other groups. Our study was the first study to evaluate the association between cadmium level and hearing impairment among the Asian population.

Cadmium is a well-known heavy metal. Participants who exposed a heavy metal may be associated with exposure to other heavy metal. Our data showed that participants with high exposure of cadmium are associated with high lead levels. Therefore, we performed multivariate analyses with lead level as a covariate. Multivariate analyses showed that cadmium level is associated with hearing impairment irrelevant to lead exposure. In addition, a previous study evaluated the association between two heavy metal and hearing impairment using similar cohort [14]. However, they did not show a positive association between cadmium and hearing impairment. Different results from similar cohort may be associated with participants’ characteristics. Previous study enrolled participants aged 20 and older, but our study enrolled participants aged 40–70. Young population or extreme elderly population can lead to bias to evaluate the two variables. Young population had extremely low prevalence of hearing impairment. Elderly population is associated with extreme high prevalence of hearing impairment and hearing impairment may be more strongly associated with other factors such as drugs, neural degeneration, or noise.

This study had several limitations to consider when interpreting the results. First, our study had a retrospective, cross-sectional design. Hence, we could not evaluate the causality between cadmium level and hearing impairment. Second, a selection bias might have been present in this study due to the exclusion of 7,560 people whose data on hearing thresholds or cadmium levels were not available. These individuals represent approximately 70.0% of the population aged 40–70 years. Third, cadmium levels were measured from blood samples and evaluated using a single measurement. The cadmium amount varies based on the recency of exposure [10]. Additionally, measuring the cadmium amount from urinary samples is a more precise method of predicting the actual cadmium level than that from blood samples. A prospective study that focuses on determining the urinary cadmium level is warranted to overcome these limitations.

In conclusion, high cadmium level quartile was associated with increased hearing thresholds and HL among the Korean adult population. Therefore, early identification of high cadmium levels and providing appropriate interventions may be helpful to reduce or prevent hearing impairment.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korea government (MSIT) (No. 2018R1C1B6007775).

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

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Successful engraftment after infusion of multiple low doses of CD34+ cells from a poorly matched sibling donor in a patient with severe aplastic anemia

Chang Dae Kum, Mi Jin Lee, Jun Eun Park

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The dose of CD34+ cells is known to influence the outcome of allogeneic peripheral blood stem cell (PBSC) and/or T-cell-depleted transplantation. A previous study proposed that $2\times10^6$ CD34+ cells/kg is the ideal minimum dose for allogeneic transplantation, although lower doses did not preclude successful therapy. In the case we present here, CD34+ cells were collected from a matched sibling donor on the day of allogeneic hematopoietic stem cell transplantation; however, the number of cells was not sufficient for transplantation. Consequently, PBSCs were collected three additional times and were infused along with cord blood cells from the donor that were cryopreserved at birth. The cumulative dose of total nuclear cells and CD34+ cells was $15.9\times10^8$ cells/kg and $0.95\times10^6$ cells/kg, respectively. White blood cells from this patient were engrafted on day 12. In summary, we report successful engraftment after infusion of multiple low doses of CD34+ cells in a patient with severe aplastic anemia.

Keywords: Aplastic anemia; CD34; Cord blood stem cell transplantation; Peripheral blood stem cell transplantation

Introduction

Severe aplastic anemia (SAA) is a rare and fatal disease characterized by immune-mediated dysfunction of hematopoietic stem cells [1]. The development of allogeneic peripheral blood stem cell transplantation (PBSCT) has dramatically increased the success rate of SAA treatment [2]. In histocompatible siblings, PBSCT is an effective treatment for SAA [3,4]. Allogeneic hematopoietic peripheral stem cell transplantation is beneficial to both donors and recipients: the donor avoids the inconvenience and risk of the collection procedure, including general anesthesia; the patient benefits from a more rapid recovery of the hematopoietic and immune systems, which reduces morbidity and promotes early discharge, compared to bone marrow transplantation [5]. It has been proposed that the minimum number of CD34+ cells in peripheral blood stem cells (PBSCs) required for successful engraftment in allogeneic hematopoietic stem cell transplantation is $2\times10^6$ cells/kg. Administration of sufficient numbers of CD34+ cells to the recipient is an important prognostic factor and is known to affect transplant-related mortality [6,7]. Here, we report a patient with SAA who received PBSCs from their human leukocyte antigen (HLA)-identical sibling. We were, however, unable to harvest a sufficient number of donor PBSCs for engraftment on the day of transplantation. As a result, multiple collections of donor PBSCs were made and infused into the recipient over 4 additional days, together with the donor’s cord blood that was cryopreserved at birth.
Case

A 6-year-old patient was hospitalized due to pancytopenia identified from the results of a complete blood cell count. At the initial diagnosis, laboratory tests showed a leukocyte count of 3.0×10^9/L, hemoglobin of 8.5 g/dL, platelet count of 19×10^9/L, absolute neutrophil (ANC) count of 0.84×10^9/L, and corrected reticulocytes at 1.43%. Furthermore, a bone marrow examination revealed hypocellularity (less than 10% marrow cellularity). After evaluation, the patient was diagnosed with moderate aplastic anemia. In accordance with the criteria of aplastic anemia, immunosuppressive therapy (IST) was performed with anti-thymocyte globulin (ATG), cyclosporine, and prednisolone (PD). The patient was treated with 3.5 mg/kg ATG for 5 days, 7.5 mg PD TID (1 mg/kg) for 5 days, and 180 mg cyclosporine-A (CSA). No IST response was observed after 6 months, and follow-up testing at this time showed a leukocyte count of 1.8×10^9/L, hemoglobin of 7.1 g/dL, platelet count of 13×10^9/L, ANC count of 0.52×10^9/L, and corrected reticulocytes at 0.40%. The patient was diagnosed with SAA, and an allogeneic hematopoietic stem cell transplant was performed 1 month later. Tests for parvovirus and cytomegalovirus (CMV) were both negative, and the patient also tested negative for congenital bone marrow failure syndromes, including Wiskott-Aldrich syndrome and Fanconi anemia.

The selected donor was the patient’s 3 year-old brother, who was HLA- and ABO-matched and blood type Rh+ O. He also tested negative for parvovirus and CMV, and there were no unusual findings in the pre-transplantation evaluation. A 10 μg/kg dose of granulocyte colony stimulating factor (G-CSF) was administered subcutaneously to the donor from day -3 to day 0, which increased the donor’s white blood cell (WBC) count to 2.09×10^9/L at day 0 (reticulocyte 2.38%, ANC 1.525×10^9/L).

The condition regimen included intravenous (IV) fludarabine (30 mg/m^2/day from day -6 to day -2), cytoxan (25 mg/kg/day from day -6 to day -3), and thymoglobin (2.5 mg/kg/day from day -3 to day -1). To prevent graft-versus-host disease (GVHD), a 5 mg/kg/day dose of IV CSA was administered beginning the day before transplantation (day -2). Additionally, mycophenolate mofetil was administered orally at a dose of 15-20 mg/kg the day after transplantation.

Hematopoietic stem cells were harvested from the donor after 4 days of G-CSF administration up to the day of transplantation, according to the allogeneic PBSCT protocol. A total volume of 180 mL (total nuclear cell [TNC] 4.89×10^6 cells/kg, CD34+ 0.36×10^6 cells/kg) was harvested, and mobilized infusion was performed during the first treatment. Since the number of CD34+ cells was much lower than the threshold value, we decided to repeat the PBSCT collection. However, despite performing a total of four PBSC harvests from the donor, the required number of CD34+ cells was not obtained (2nd PBSCT: TNC 3.56×10^6 cells/kg, CD34+ 0.29×10^6 cells/kg; 3rd PBSCT: TNC 4.90×10^6 cells/kg, CD34+ 0.21×10^6 cells/kg; 4th PBSCT: TNC 2.39×10^6 cells/kg, CD34+ 0.06×10^6 cells/kg). As a result, we also administered the donor’s cord blood cells that were frozen and stored, followed by cord blood infusion with 0.14×10^6 cells/kg TNC and 0.03×10^6 cells/kg CD34+ cells. The total cell count for the five injections was 15.88×10^6 cells/kg TNC and 0.95×10^6 cells/kg CD34+ (Table 1).

For engraftment, IV administration of G-CSF 250 μg (300 μg/m^2) was started on day 4. Twelve days after the first transplantation, WBC engraftment was achieved with a mean neutrophil count >0.5×10^9/L. Platelet engraftment without transfusion for over 2 weeks (platelet count >20×10^9/L) was achieved on day 21. The donor chimerism result was completely changed when measured 1 month after the first stem cell infusion. After transplantation, the patient showed no signs of acute/chronic GVHD and was discharged from the hospital. The patient maintained complete remission; bone marrow examination results revealed cellularity above 50%, and the donor chimerism value was maintained for a full year.

Table 1. Five transplantation in 5 days, including cord blood from the donor.

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Date of infusion (day)</th>
<th>TNC (x10^6/kg)</th>
<th>CD34+ cell (x10^6/kg)</th>
<th>Stem cell source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>0</td>
<td>4.89</td>
<td>0.36</td>
<td>PB</td>
</tr>
<tr>
<td>2nd</td>
<td>1</td>
<td>3.56</td>
<td>0.29</td>
<td>PB</td>
</tr>
<tr>
<td>3rd</td>
<td>2</td>
<td>4.90</td>
<td>0.21</td>
<td>PB</td>
</tr>
<tr>
<td>4th</td>
<td>4</td>
<td>2.39</td>
<td>0.06</td>
<td>PB</td>
</tr>
<tr>
<td>5th</td>
<td>5</td>
<td>0.14</td>
<td>0.03</td>
<td>CB</td>
</tr>
<tr>
<td>Total cell count</td>
<td>15.88</td>
<td>0.95</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TNC, total nuclear cell; PB, peripheral blood; CB, cord blood.

Discussion

SAA is a heterogeneous disorder of bone marrow stem cells and/or their microenvironment, and can be due to various mechanisms, including autoimmune processes and viral infection [4]. If treated inappropriately, SAA can be a potentially fatal disorder in children. The main approaches to SAA therapy are hematopoietic stem cell transplantation and IST, and for children the current algorithm recommends transplantation, if there is a matched sibling donor. In the absence of a matching donor, IST...
using a combination of ATG and cyclosporine is the first choice of treatment [8-10].

The importance of CD34+ cell dose in PBSCT has been clearly demonstrated in autologous or homogeneous peripheral blood hematopoietic stem cell transplantation. CD34 is a type I transmembrane protein expressed by hematopoietic stem cells, and CD34+ cells have the ability to differentiate into all bone marrow cell lineages. Clinically, CD34+ cells are considered equivalent to pluripotent stem cells, with self-renewing capabilities. The dose of injected CD34+ cells is known to be an important factor in autoimmune and allogeneic stem cell transplantation success [11]. The infusion of large numbers of CD34+ cells in PBSCT is thought to accelerate hematopoietic engraftment and reduce graft-related morbidity [12,13].

In a previous study, Singhal et al. recommended a CD34+ cell dose of 2×10^6 cells/kg as the minimum threshold for allogeneic sibling blood or marrow stem cell transplantation [7]. Bittencourt et al. recommended a CD34+ cell dose of 3×10^6 cells/kg as optimal for allogeneic BM transplantation in terms of faster engraftment and decreased treatment-related mortality [6]. In 2010, Islam et al. showed that infusion of less than 2×10^6 CD34+ cells/kg was associated with an increased incidence of graft failures, a higher incidence of bacterial infection, and a delay in neutrophil engraftment [11].

In the present case, we injected 0.36×10^6 cells/kg CD34+ cells, which is much lower than the previously proposed minimal threshold of 2×10^6 CD34+ cells/kg. We administered four PBSCT injections with 0.92×10^6 cells/kg CD34+ cells, followed by cord blood infusion with 0.14×10^6 cells/kg TNC and 0.03×10^6 cells/kg CD34+ cells. Despite the fact that these values were lower than the recommended threshold value, ANC engraftment was successful after 12 days without any additional abnormalities, such as GVHD infection. The result of bone marrow and polymerase chain reaction with short tandem repeat markers showed that the patient maintained full donor chimerism at their 1 month and 1 year follow-up examinations. After the first transplantation, it took 12 days to engraft the ANC, 8 days after the fourth PBSC infusion, and 7 days after the cord blood transplant. Additionally, de la Rubia et al. showed that the median time taken to achieve engraftment with neutrophil counts >0.5×10^9/L was 12 days (range, 9–13 days) [14]. From this, the first peripherally infused stem cells (CD34+ 0.36×10^6 cells/kg) were considered to have a major influence on engraftment in the present case.

In addition, the donor cord blood cell infusion, which was performed last, is known to contain mesenchymal stem cells (MSCs), which improve the recovery of hematopoietic function and prevents GVHD following hematopoietic stem cell transplantation (HSCT). MSCs are a type of adult stem cell found in many tissues and organs. They are capable of self-renewal, multiple screening, and regulation of immune function. MSCs are progenitors of bone marrow stroma, and MSCs isolated from bone marrow, blood, and cord blood have been shown to promote engraftment after hematopoietic stem cell transplantation. Moreover, MSCs facilitate engraftment of neutrophils and platelets, and contribute to 100% donor chimerism. MSCs in HSCT can promote stem cell transplantation, improve hematopoietic function recovery, and prevent GVHD [15]. In the present case, it is possible that MSCs facilitated the engraftment of neutrophils and platelets contained in the administered peripheral blood and cord blood stem cells. Additionally, cord blood stem cell infusion may have supplemented the otherwise small CD34+ cell dose. The reason for the insufficient number of CD34+ cells harvested from the donor is not clear. The donor’s body weight was 16.9 kg, which is less than the patient, and could be one of the causes of the insufficient harvest. We attempted to further evaluate the donor, but his mother refused.

In conclusion, we report successful engraftment after infusion of multiple low doses of CD34+ cells from a poorly matched sibling donor in a patient with SAA.

**Conflicts of interest**

No potential conflicts of interest relevant to this article was reported

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**References**


Surgical treatment of esotropia and unilateral ptosis in a patient with Cornelia de Lange syndrome

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Cornelia de Lange syndrome (CdLS) is a rare multisystemic disorder that is characterized by mental retardation, prenatal and postnatal growth retardation, limb anomalies, and distinctive facial features, which include arched eyebrows that often meet in the middle (synophrys), long eyelashes, low-set ears, small and widely spaced teeth, and a small and upturned nose. Ophthalmic manifestations include long eyelashes, nasolacrimal duct obstruction, myopia, ptosis, and strabismus. There has been no report of surgical treatment for esotropia and unilateral ptosis in patients with CdLS in Korea. I report a patient with CdLS who underwent surgical treatment for esotropia and unilateral ptosis with a good surgical outcome.

Keywords: Cornelia de Lange Syndrome; Strabismus; Ptosis; Surgery

Introduction

Cornelia de Lange syndrome (CdLS) is a rare multisystemic disorder that is characterized by mental retardation, prenatal and postnatal growth retardation, limb anomalies, and distinctive facial features, which include arched eyebrows that often meet in the middle (synophrys), long eyelashes, low-set ears, small and widely spaced teeth, and a small and upturned nose [1-3]. The clinical features vary widely among affected individuals and range from relatively mild to severe. Ophthalmic manifestations include long eyelashes, nasolacrimal duct obstruction, myopia, ptosis, and strabismus [4,5]. There has been no report of surgical treatment for esotropia and unilateral ptosis in patients with CdLS in Korea. I report a patient with CdLS who underwent surgical treatment for esotropia and unilateral ptosis with a good surgical outcome.

Case

Informed consent was obtained from the patient's parent. A 5-year-old girl was referred to our ophthalmology department for surgical treatment of esotropia and unilateral ptosis of the right eye. She had been previously diagnosed with CdLS at another clinic and had undergone regular follow-up. She had been born at 36 weeks of gestation with a birth weight of 1,900 g. She had the typical facial features of CdLS, psychomotor delay, and intellectual disability (Fig. 1). We were unable to measure the visual acuity due to poor cooperation. The fixation of the right eye was weaker compared with that of the left eye. She also had ptosis, which covered the visual axis of the right eye. The extraocular examination showed 50 prism diopters of esotropia with abduction limitation of the right eye (Fig. 2A). Nystagmus was not observed. The refractive errors were -0.50 diopters (D) in both eyes. The fundus examination revealed peripapillary pigmentation of the right eye and a normal appearance of the left eye. The forced duction test was performed under general anesthesia; it did not reveal any restriction of the medial rectus in the right eye. Exploration showed that the lateral rectus muscle had a normal appearance, and it did not have any restriction.
A 7-mm resection of the lateral rectus and 6-mm recession of the medial rectus were performed on the right eye without augmentation. The frontalis sling procedure was performed for the treatment of right ptosis. Four months later, the patient had stable ocular alignment with good eyelid position (Fig. 2B).

Discussion

CdLS occurs sporadically in most patients [2,5]. The exact etiology of CdLS is still unknown, but five genes have been reported to be associated with CdLS; the mutations of these genes comprise the underlying defect in 70% of the patients [2,6]. The initial diagnosis of CdLS is based on the clinical features, with specific signs, symptoms, and developmental characteristics forming the main criteria. Clinical diagnostic criteria have been proposed and include long eyelashes, ptosis, tear duct malformation or blepharitis, myopia ≥ -6.00 D, and a major eye malformation or peripapillary pigmentation as ophthalmic features [2]. Therefore, an ophthalmic examination can be helpful in the diagnosis of CdLS in an atypical case. In the present case, the patient had large-angle esotropia and severe unilateral ptosis. Compared with strabismus, ptosis is a relatively common clinical feature. Wygnanski-Jaffe et al. have reported the ophthalmic features in 120 patients with CdLS [7]. In their largest series, 10% of patients (11/113) had esotropia and 44% of patients (41/117) had varying degrees of ptosis, ranging from mild cosmetic changes to functional obstruction of the visual axis. Nallasamy et al. have evaluated the genotype-phenotype correlation with regard to the severity of ophthalmic findings in patients who had been previously screened for mutations in the Nipped-B-like gene [5]. In their study, nearly one-half of the included patients (25/54) had ptosis, and its severity was increased in patients who had truncating mutations compared to those who had missense mutations. Only 2 of the 15 patients with strabismus required surgical treatment. Because patients with CdLS have short horizontal palpebral fissures [7], it is sometimes difficult to get a clear surgical field during strabismus surgery, especially during medial rectus muscle surgery. A surgeon should take these into consideration during surgical planning.

Patients with CdLS have many ophthalmic problems, such as ptosis, strabismus, refractive errors, and nasolacrimal duct obstruction [5,7,8]. The treatment strategy should be based on each patient’s symptoms. The surgical correction of ptosis and strabismus can improve the quality of the life of an affected patient. This report should help surgeons to consider surgical treatment of ptosis and strabismus in patients with CdLS.

Conflicts of interest

No potential conflicts of interest relevant to this article was reported.
Patient consent

Patient’s guardians provided written consent for the use of her images.

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References

Imatinib-induced hepatitis treated by corticosteroids in a patient with metastatic gastrointestinal stromal tumor

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Introduction

Imatinib mesylate is currently used as the first-line treatment for metastatic gastrointestinal stromal tumors (GISTs). Imatinib-induced hepatotoxicity in patients with GIST is very rare. Its features vary from subclinical elevation of serum aminotransferase to clinically apparent acute hepatitis, which is associated with immunologic reactions. Imatinib-induced hepatotoxicity with autoimmune-like features can be treated by the discontinuation of imatinib mesylate and the administration of oral steroids. Here, we report a case of late-onset imatinib-induced hepatitis with autoimmune-like features in a patient with metastatic GIST, which was improved by oral corticosteroids.

Case

A 55-year-old man was referred to our department with deterioration of liver function. He had undergone segmental resection of the small bowel and peritonectomy for metastatic GIST, which had caused massive hematochezia 10 months ago. The tumor had spread into the omentum and the pelvic cavity with scattered nodules. The pathology results indicated the co-expression of CD113 and CD34 spindle cells, with a mitotic index of 4 per 50 high-power fields. After the surgery, palliative chemotherapy with imatinib mesylate 400 mg daily was initiated. After 10 months of imatinib therapy, the patient was referred to our department owing to elevated aminotransferase level without any specific symptoms for several months. He denied any causal alcohol consumption or medication. His physical examination was normal. The laboratory tests revealed the following values: white blood cell count, 4,770/µL; eosinophil count, 467/µL (range, <500/µL); hemoglobin, 13.8 g/µL;
platelet count, 184 K/µL; total protein, 6.34 g/dL; albumin, g/dL; total bilirubin (TB), 1.59 mg/dL; direct bilirubin, 0.31 mg/dL; aspartate aminotransferase (AST), 239 IU/L; alanine aminotransferase (ALT), 393 IU/L; alkaline phosphatase, 194 IU/L (range, 30–120 IU/L); gamma glutamyl transferase, 52 IU/L (range, <50 IU/L); blood urea nitrogen, 12 mg/dL; creatinine, 1 mg/dL; and international normalized ratio, 1.06. Serologic markers for viral hepatitis, including hepatitis A virus, hepatitis B virus, and hepatitis C virus, were negative. There was no evidence of acute viral infection, and tests for Epstein-Barr virus, cytomegalovirus, and herpes virus were not performed. To exclude autoimmune hepatitis (AIH) and primary biliary cholangitis, additional serologic tests were conducted, including anti-nuclear antibody, anti-smooth muscle antibody, anti-mitochondria antibody, immunoglobulin G (IgG), IgA, IgM, anti-neutrophil cytoplasmic antibody, and anti-liver kidney microsomal type 1 antibody, and all results were within the normal ranges. Computed tomography (CT) of the abdomen revealed no evidence of recurrence of GIST compared with previous CT scan (Fig. 1). After immediate discontinuation of imatinib therapy, supportive treatment, including 300 mg ursodeoxycholic acid daily and nutritional support with high calorie and protein, was initiated. Despite 3 weeks of supportive treatment, serum levels of AST, ALT, and TB increased up to 255 IU/L, 470 IU/L, and 2.07 mg/dL, respectively. The R factor for liver injury was 7.6, which was equivalent to hepatocellular liver injury. The Roussel Uclaf Causality Assessment Method score was 6, which

![Fig. 1. Abdominal computed tomography reveals no evidence of recurrence of GIST from upper abdomen (A) to lower abdomen (B) after segmental resection of small bowel and peritonectomy for metastatic GIST. GIST, gastrointestinal stromal tumor.](https://doi.org/10.12701/yujm.2019.00115)

![Fig. 2. Histological findings of the liver. (A) There is interface hepatitis with inflammatory cells infiltrations of lymphocytes and plasma cells (arrows) in portal and periportal area (hematoxylin and eosin stain, ×100). (B) The centrilobular necrosis is present, with golden-brown colored ceroid pigment-laden Kupffer cells (arrows) and shrunken, eosinophilic apoptotic hepatocytes (arrow head) (hematoxylin and eosin stain, ×200).](https://doi.org/10.12701/yujm.2019.00115)
indicated that drug-induced liver injury (DILI) was probable.

To exclude the possibility of DILI, a percutaneous liver biopsy guided by ultrasound was performed. The pathologic findings revealed centrilobular necrosis and mild interface hepatitis, consistent with DILI with suspicious autoimmune-like features (Fig. 2). The Revised Original Score for AIH for pretreatment was 10, which indicated that AIH was probable.

As we could not completely rule out the relationship with autoimmunity, we decided to administer steroids. The patient was treated with 30 mg prednisolone daily. After 2 weeks, his laboratory test results were improved: AST, 40 IU/L; ALT, 132 IU/L; and TB, 0.87 mg/dL. Subsequently, prednisolone was tapered, to 15 mg daily for 2 weeks. After 4 weeks, his laboratory test results were normal: AST, 29 IU/L; ALT, 132 IU/L; and TB, 0.91 mg/dL (Fig. 3). A reduced dose of imatinib mesylate (300 mg daily) was restarted without further deterioration of liver function over a follow-up period of 4 months. In addition, Revised Original Score for AIH for post-treatment was 12, which indicated that AIH was probable.

Discussion

The clinical manifestations of imatinib-induced hepatotoxicity are varied in patients with GIST (Table 1). In our patient, grade 3 ALT elevation first occurred 10 months after the administration of imatinib. The onset period of imatinib-induced hepatotoxicity is reported to be between 11 days and 13 months [4,6]. Serum levels of aminotransferase are usually elevated within the first 2–3 months after imatinib administration, and are improved within 4 weeks after discontinuation [4,7]. In addition, the clinical features of imatinib-induced hepatotoxicity in patients with GIST range from transient elevation of serum aminotransferase to acute liver injury, which may cause cirrhosis [2,4,8].

Although the pathogenic mechanisms of imatinib-induced hepatotoxicity are not fully understood, they may be associated with idiosyncratic hypersensitivity reactions to the drug in susceptible patients [9]. The drug metabolism pathway in the liver is divided into four phases, phase 0 to phase 3 [10,11]. In phase 0, the drug is transported from the blood to the liver via the carrier-mediated uptake system, including soluble carrier (SLC21/SLCO) [11]. The drug transported into the liver is metabolized in phase 1 (oxidation via the enzyme cytochrome P [CYP] 450) and phase 2 (conjugation via mainly UDP-glucuronosyltransferase) [10]. In phase 3, conjugated metabolites are excreted into the cells by active transporter pumps using multidrug resistance-associated protein 2 and multidrug resistance protein 1/P-glycoprotein [11].

Imatinib mesylate is metabolized mainly by CYP450 enzymes, such as CYP3A4, CYP2C9, and CYP2D [2]. Drugs and foods that interfere with CYP450 enzymes, such as erythromycin, roxithromycin, itraconazole, and grapefruit juice, may elevate the serum concentration of imatinib metabolites, which may cause hepatotoxicity [2]. The pregnane X receptor and constitutive androstane receptor play an important role in drug interactions, acting as transcriptional regulators of CYP 450 [12].

In addition, the histopathologic findings of imatinib-induced hepatitis include diverse manifestations, including periportal inflammation, focal necrotic hepatocytes with mixed lymphocytes, neutrophil infiltration, extensive centrilobular hepatic necrosis, and severe cytoltytic acute hepatitis [1,2].

Most cases of hepatotoxicity are resolved by reduction or discontinuation of the medication. In one case, steroids reversed

![Fig. 3. Clinical course of the patient. ALT, alanine aminotransferase.](https://doi.org/10.12701/yujm.2019.00115)

**Table 1. Imatinib-induced hepatotoxicity in patients with gastrointestinal stromal tumor**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age/sex</th>
<th>Dose (mg)</th>
<th>Time to hepatotoxicity</th>
<th>Type of hepatotoxicity</th>
<th>Steroid initial dose (mg/day)</th>
<th>Rechallenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pariente et al. [1]</td>
<td>71/F</td>
<td>400 then 300</td>
<td>7 wk</td>
<td>Cytolytic hepatitis</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Tonyali et al. [2]</td>
<td>53/F</td>
<td>400</td>
<td>10 wk</td>
<td>Acute liver failure</td>
<td>PD 40</td>
<td>Sunitinib</td>
</tr>
<tr>
<td>Seidal et al. [3]</td>
<td>49/M</td>
<td>400</td>
<td>6 mon</td>
<td>Liver cirrhosis</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Yachoui [4]</td>
<td>46/F</td>
<td>400</td>
<td>11 d</td>
<td>Acute hepatitis</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Saif et al. [14]</td>
<td>65/M</td>
<td>400 then 200</td>
<td>2 mon</td>
<td>Gilbert’s syndrome</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Our case</td>
<td>55/M</td>
<td>400 then 300</td>
<td>10 mon</td>
<td>Acute hepatitis</td>
<td>PD 30</td>
<td>Yes</td>
</tr>
</tbody>
</table>

PD, prednisolone.
imatinib-induced immune hepatitis, as characterized by persistent elevations of autoantibodies, including anti-nuclear antibody and double-strand antibodies, after discontinuation of imatinib for 2 weeks [6]. In addition, despite no clear immunologic features, imatinib-induced hepatotoxicity was improved after the administration of oral steroids [2,5]. In our case, despite discontinuation of imatinib for 3 weeks, laboratory results were exacerbated, with elevations in ALT (grade 4) and bilirubin (grade 2), without definite evidence of AIH. However, liver biopsy revealed acute DILI with suspicious autoimmune features, including interface hepatitis, infiltration of inflammatory cells, and focal necrosis of hepatocytes. In addition, the revised Original Scores for AIH for pre- and post-treatment were 10 and 12, respectively. These two scores indicate that autoimmune features are probable. Although the mechanism of the corticosteroids is still unclear, it is presumed to be related to anti-inflammatory effects [5]. This is supported by our case, in which the patient improved after treatment of drug-induced, autoimmune-like hepatitis with corticosteroids.

For the imatinib rechallenge, a 25% dose reduction was used after confirmation of normal blood levels of AST, ALT, and TB.

Drug rechallenge is associated with 13% mortality, and considerable comorbidity [13]. However, in clinical practice, the risk-benefit stratification for drug rechallenge is important and re-administration may be undertaken carefully in the following cases: if continuous treatment, such as that needed for cancer, is required; there is no safer alternative treatment; if the benefit is judged to be greater than the risk; or if the patient consents after sufficient explanation of the risk of adverse event [13]. In our case, as all these conditions were satisfied, we re-administered imatinib. In addition, the dose reduction and dosage at the time of re-administration of the drug are not clearly defined, and individualization and treatment tailoring through close clinical follow-up is required.

In conclusion, the appropriate administration of oral steroids with prompt discontinuation of imatinib may be helpful for patients with imatinib-induced, autoimmune-like hepatitis.

Conflicts of interest

No potential conflicts of interest relevant to this article was reported.

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Rapid progression from trochlear nerve palsy to orbital apex syndrome as an initial presentation of advanced gastric cancer

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Introduction

The metastasis to the orbit and ocular adnexa is rare and occurs less frequently than metastasis to the eye [1-3]. However, the improved survival of patients with common cancers together with the progressive aging of the population has led to a higher incidence of patients living with metastatic disease in unusual sites, such as the orbit and ocular adnexa [1]. The most cases with orbital metastases have been reported in patients with a prior established diagnosis of cancer and widespread systemic involvement. However, ocular symptoms can be developed as an initial presentation of cancer in patients without cancer history. We report a case of rapid progression from trochlear nerve palsy to orbital apex syndrome as an initial presentation of advanced gastric cancer.

Keywords: Diplopia; Metastasis; Optic neuropathy; Stomach neoplasms; Trochlear nerve palsy

Case

A 56-year-old patient visited the ophthalmic department for vertical diplopia for 1.5 months. The symptom did not have diurnal variation or relation to fatigue. He reported mild painful sensation of the left periocular area. There was no ocular injection, chemosis, moving pain, and visual disturbance. He did not report any other systemic disease. He had no prior history of strabismus or objective diplopia. Visual acuity was 20/20 at both eyes. The pupil showed normal response to both light and near stimulation. He demonstrated 4 prism diopters (PD) hypertropia of the left eye at the primary gaze. The Bielschowsky head tilt test revealed 8 PD of left hypertropia on left tilt and orthotropia on right tilt. The fundus examination showed normal appearance of optic disc and no abnormal torsional finding in both eyes. Acquired isolated trochlear nerve palsy of the left eye was suspected based...
on the ocular motility findings. Besides the ocular symptoms, he complained of lower back pain since 2 months. The magnetic resonance imaging (MRI) with magnetic resonance angiography of brain, performed at another clinic, revealed no definitive abnormal findings. Serologic tests were conducted to evaluate the cause of diplopia. The initial laboratory results showed the following abnormal values: white blood cell count of 15,750/μL (range, 4,000-10,000/μL), blood urea nitrogen of 32 mg/dL (range, 8-23 mg/dL), alkaline phosphatase (ALP) of 3,828 IU/L (range, 30-120 IU/L), aspartate alanine transferase of 41 IU/L (range, 10-35 IU/L), and alanine aminotransferase of 96 IU/L (range, 0-46 IU/L).

At the follow-up visit 1 week later, he reported that the visual acuity of left eye had suddenly deteriorated to hand motion. The pupil of the left eye showed relative pupillary defect at light stimulation, but fundus had normal appearance. The follow-up MRI demonstrated inhomogeneous enhancement surrounding the optic nerve at the orbital apex through the cavernous sinus of the left eye, which was suspected to have had progressed compared to the previous neuroimaging (Fig. 1). The computed tomography of the chest and abdomen were performed to evaluate the cause of elevated liver enzymes and ALP and revealed diffuse osteoblastic and osteolytic metastases in visible sections of skeleton, including the thoracolumbar spines, rib cage, and shoulder girdles. The whole body bone scan revealed multiple hot uptake in the skull, whole spines, both scapulas, sternum, both humeri, pelvic bone, and both femurs. The lesion in the orbital apex of the left eye was confirmed by positron emission tomography (Fig. 2). To locate the primary cancer of bone metastasis, we performed the esophagogastroduodenoscopy (EGD) and colonoscopy. The EGD revealed a 7×5 cm sized ulceroinfiltrating mass from the proximal body to angle of stomach. The biopsy of the mass revealed a signet ring cell carcinoma. He was finally diagnosed with gastric cancer with multiple bone and lymph node metastases.

**Discussion**

Most patients with metastatic tumors to the orbit already have a known diagnosis of a primary carcinoma with other evidence of metastases at the time of occurrence of ocular symptoms. However, ocular symptoms can present as the first sign of systemic cancer [1-3]. The clinical manifestations of orbital metastasis are variable and include diplopia, proptosis, decreased vision, increased orbital

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**Fig. 1.** Axial magnetic resonance imaging of the brain with contrast showing enlargement and enhancement of the left orbital apex (arrow) through the cavernous sinus (A). This lesion (arrow head) was also suspected in previous neuroimaging performed 2 months earlier (B).
pressure, and limited ocular motility [1-3]. Generally, these clinical presentations are unrelenting, have a rapid onset, and entail a disproportionate number of symptoms compared to other space-occupying orbital lesions [1,4].

These characteristics are consistent with the ocular findings of our case. Our patient initially developed vertical diplopia caused by trochlear nerve palsy. The visual deterioration of the left eye developed in a short period and was caused by optic neuropathy. The follow-up neuroimaging performed 2 months after the initial examination demonstrated a lesion around the orbital apex through the cavernous sinus which was not suspected definitively in previous study. This rapid progression of ocular symptoms may
be attributed to the anatomical location of lesion between the superior orbital fissure, through which the trochlear nerve passes, and the optic canal, through which the optic nerve passes at the orbital apex [4,5].

Ipsilateral cranial nerve dysfunction involving a combination of oculomotor nerve, trochlear nerve, trigeminal nerve, abducens nerve, and sympathetic fibers is the hallmark of ophthalmoplegia that is secondary to a cavernous sinuses lesion [4-7]. The superior orbital fissure syndrome is often applied to lesions located immediately anterior to the orbital apex, result to cranial nerve palsies absence of optic nerve damage. The orbital apex syndrome has been described as a syndrome involving damage to the multiple cranial nerve with optic nerve dysfunction [8]. These terms define the precise anatomic location of lesions. However, it is often impossible to distinguish cavernous sinus lesions clinically from those involving the superior orbital fissure. The superior orbital fissure, orbital apex, and cavernous sinus are all contiguous, patients with superior orbital fissure syndrome can subsequently develop orbital apex and cavernous sinus pathology like our case [8].

Any cancer that can spread via the hematogenous route can metastasize to the orbit and ocular adnexal structures [1]. Breast cancer has been reported as the most common primary cancer of orbital metastasis in various large studies [1]. However, recent studies have shown that the most common primary cancer of orbital metastasis is nasopharyngeal carcinoma in southern China and lung cancer in Japan [2,3]. These results may be attributed to differences in epidemiology and incidence of the primary cancers. The prognosis for survival in patients with orbital metastasis remains poor. Future advancements in combined treatment modalities may improve quality of life and preserve ocular function. Prompt recognition may help in the detection of an unrecognized primary systemic cancer and allow for early treatment. Yan and Gao reported that orbital metastasis was the first sign of systemic cancer in approximately half of the included patients [3]. Therefore, ocular examination may have a crucial role in the detection and staging of a previously unsuspected primary cancer. Although orbital metastasis is rare, it should be considered in the differential diagnosis of ocular motility disorder and optic neuropathy.

**Conflicts of interest**

No potential conflicts of interest relevant to this article was reported.

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