REVIEW ARTICLE

Alpha-melanocyte stimulating hormone in ghrelin-elicited feeding and gut motility 87
   Hsien-Hao Huang, Chih-Yen Chen

ORIGINAL ARTICLES

Halofuginone protects HUVECs from H₂O₂-induced injury by modulating VEGF/JNK signaling pathway 92
   Bin He, Guo-Hua Fu, Xian-Feng Du, Hui-Min Chu

Elevated serum ferritin level associated with hepatic steatosis and fibrosis in hepatitis C virus–infected patients 99
   Batbold Batsaikhan, Gantsetseg Gantumur, Ching-I Huang, Ming-Lun Yeh, Chung-Feng Huang, Zu-Yau Lin,
   Shinn-Cherng Chen, Jee-Fu Huang, Ming-Lung Yu, Wan-Long Chuang, Jin-Ching Lee, Chia-Yen Dai

Retrospective analysis of endoscopic management of foreign bodies in the upper gastrointestinal tract of adults 105
   Chung-Ying Lee, Bi-Zhen Kao, Chia-shin Wu, Ming-Yao Chen, Hsi-Yuan Chien, Li-Wei Wu, Sheng-Tsai Lin,
   Yu-Hsin Tai, Hwai-Jeng Lin

Identification of a homozygous BBS7 frameshift mutation in two (related) Chinese Miao families with Bardet-Biedl Syndrome 110
   Tao Shen, Jian-Mei Gao, Tao Shou, Li Li, Jin-Ping Zhang, Qian Zhao, Xin-Min Yan

Anatomic mapping of the internal spermatic vein via subinguinal varicocelectomy with intraoperative vascular Doppler ultrasound 115
   Yu-Cing Juho, Sheng-Tang Wu, Chien-Chang Kao, En Meng, Tai-Lung Cha, Dah-Shyong Yu

The effect of high-dose nitroglycerin on the cerebral saturation and renal function in cardiac surgery: A propensity score analysis 120
   Ying-Hsuan Tai, Hsiang-Ling Wu, Fu-Wei Su, Kuang-Yi Chang, Cheng-Hsiung Huang, Mei-Yung Tsou,
   Chih-Cheng Lu

The usefulness of prophylactic use of acetazolamide in subjects with acute mountain sickness 126
   Pin-Hsi Hung, Fang-Chi Lin, Han-Chen Tsai, Heng-Sheng Chao, Chung-Wei Chou, Shi-Chuan Chang

Prognostic factors related to intratumoral hemorrhage in pediatric intracranial germ cell tumors 133
   Ju-Ting Chen, Han-Jui Lee, Yi-Wei Chen, Muh-Li Liang, Hsin-Hong Chen, Yi-Yen Lee, Jiing-Feng Lirng,
   Chao-Bao Luo, Feng-Chi Chang, Wan-Yuo Guo

Predictors of subsequent pregnancy in women who underwent laparoscopic cornuostomy or laparoscopic wedge resection for interstitial pregnancy 138
   Pei-Ling Chen, Ho-Hsiung Lin, Sheng-Mou Hsiao

The mid-term outcome of dialysis-dependent patients undergoing primary total knee arthroplasty and total hip arthroplasty: A retrospective study 143
   I-Ning Lo, Shang-Wen Tsai, Po-Kuei Wu, Cheng-Fong Chen, Ming-Chau Chang, Wei-Ming Chen
The impact on outcomes by using thiotepa in tandem transplant for pediatric high-risk embryonal brain tumors
  Hsiu-Ju Yen, Ting-Yen Yu, Chih-Ying Lee, Gun-Yi Hung, Tzeon-Jye Chiou, Hsin-Hung Chen, Yi-Yen Lee, Muh-Lii Liang, Yi-Wei Chen  
  148

Multisection computed tomography: Results from a Chinese survey on radiation dose metrics
  Dan-Dan Zhou, Pengfei Sun, Zhifang Jia, Wanan Zhu, Guang Shi, Boyu Kong, Haifeng Wang, Huimao Zhang  
  155

Health-related quality of life in children and adolescent with different types of scoliosis: A cross-sectional study
  Po-Cheng Hsu, Chi-Kuang Feng, Shou-Hsien Huang, Jan-Wei Chiu, Chen-Liang Chou, Tsui-Fen Yang  
  161
Alpha-melanocyte stimulating hormone in ghrelin-elicited feeding and gut motility

Hsien-Hao Huang, Chih-Yen Chen

1. INTRODUCTION

Proopiomelanocortin (POMC) is an important precursor protein in the central melanocortin system. Immuno-histochemical studies have revealed that POMC precursor is most abundant in the arcuate nucleus of the hypothalamus (ARC) and the nucleus of the solitary tract (NTS) in the brainstem. POMC is a large molecule that is cleaved into several biologically regulatory peptides, termed melanocortins. These include \( \alpha \), \( \beta \), and \( \gamma \)-melanocyte stimulating hormones (MSHs) and adrenocorticotropin (ACTH). These melanocortins exert their activity by binding to a family of melanocortin receptors (MCRs). Five receptor subtypes with specific and distinct affinities for MSH/ACTH have been cloned: MC1, MC2 (or ACTH), MC3, MC4, and MC5 receptor. \( \alpha \)-MSH displayed high affinity to the MC1 receptor, distinct from the other MC receptors. \( \alpha \)-MSH displayed high affinity to the MC1 receptor, distinct from the other MC receptors.

1.1. MC1 receptor

The MC1 receptor was the MCR to be cloned and expressed in melanocytes and melanoma cells and in a limited brain area. The MC1 receptor is confined to the hypothalamus.

1.2. MC2 receptor

The MC2 receptor is abundant in the adrenal gland. It is not present in the hypothalamus and pituitary, based on the absence of detectable MC2 receptor mRNA. The MC2 receptor does not couple with MSH peptides but has high affinity with ACTH. Thus, MC2 receptor has been identified as the ATCH receptor and regulates steroid production in the adrenal gland.

1.3. MC3 receptor

The MC3 receptor is predominantly expressed in the brain (the arcuate nucleus), placenta, gut tissue, and human heart. MC3 receptor knockout mice display metabolic syndrome evident as decreased fat/carbohydrate oxidation, reduced energy expenditure, and increasing adipose mass without increased food intake or weight gain. MTII is a potent MR agonist for both the MC3 receptor and MC4 receptor. MTII does not induce anorectic action and decreases food intake in MC4 receptor knockout mice. These findings support the speculation that MC3 receptor has limited importance in mediating MTII-induced anorectic action and decreased food intake. Owing to the lack of MC3 receptor specific ligands, the role of MC3 receptor in maintaining metabolic homeostasis is still obscure and requires further investigation.

1.4. MC4 receptor

The MC4 receptor is found mainly in the central nervous system, but is also expressed throughout the brain, including the thalamus, hypothalamus, cortex, and brain stem as well as in the spinal cord. Deletion of the gene encoding MC4 receptor...
results in hyperphagia, increased food consumption, and profound obesity.\textsuperscript{21} MC4 receptor knockout mice do not respond to the anorectic action and reduced food intake of MTII.\textsuperscript{18,19} Mutation or deletion of the MC4 receptor is associated with obese, hyperphagic, and hyperinsulinaemic phenotypes.\textsuperscript{21–23} The MC4 receptor is expressed in the dorsal motor nucleus of the vagus within the hindbrain,\textsuperscript{20} which is the site of parasympathetic vagal efferent nerves that regulate the gastrointestinal system.\textsuperscript{24} Intracerebroventricular (ICV) injection of specific MC4 receptor antagonists (HS014, HS024, and HS028) significantly increases the food intake.\textsuperscript{12,25} These findings indicate that signaling of the MC4 receptor regulates food intake and body fat mass. MC4 receptors are up-regulated in food-limited rats but down-regulated in diet-induced obese rats.\textsuperscript{26} Subtle alterations in MC4 receptors function and density may be essential in the regulation of weight control.\textsuperscript{27}

1.5. MC5 receptor

The MC5 receptor is expressed abundantly in a variety of peripheral tissues, such as skeletal muscle, lung, stomach, spleen, kidney, liver, and testis.\textsuperscript{7,28,29} The expression of MC5 receptor in the brain is inconsistent, being very low in the rat\textsuperscript{7} but abundant in the mouse.\textsuperscript{28,29} The MC5 receptor has a role in the regulation of exocrine gland function.\textsuperscript{30}

1.6. α-MSH

α-MSH is the principle identified agonist in the brain.\textsuperscript{11} Immunocytochemical staining data indicate that α-MSH strongly activates the hypothalamus, thalamus, brainstem,\textsuperscript{31} the arcuate region of the hypothalamus,\textsuperscript{32} and paraventricular nuclei of hypothalamus neurons,\textsuperscript{5,15} which send axonal projections to many areas of the limbic system and brain stem.\textsuperscript{32} α-MSH can also induce a cAMP response in the cellular production of MC1, MC3, MC4, and MC5 receptors.\textsuperscript{12} The MC3 and MC4 receptors have been cloned and primarily expressed in the brain,\textsuperscript{5,6,9} which has revealed the avid affinity of α-MSH for both receptors.\textsuperscript{8,32} By acting on MC3 and MC4 receptors following ICV injection, α-MSH is very effective in suppressing food intake.\textsuperscript{23,33–36} If α-MSH is persistently delivered into the hypothalamus in rats, the suppression of food intake and decreased body weight will persist.\textsuperscript{39} α-MSH is considered as an agonist of MC3 and MC4 receptors and a stable agonist concerning the modulation of food intake. This view is compatible with the finding that underweight and normal-weight children have higher circulating plasma α-MSH levels compared with obese children.\textsuperscript{40}

2. EFFECTS OF α-MSH IN GHRELIN-ELICITED FOOD INTAKE

2.1. Ghrelin elicits food intake

Ghrelin is an endogenous ligand for growth hormone (GH) secretagogue receptors (GHS-R). It potently stimulates GH secretion and ghrelin-immunoreactive neurons in the hypothalamic arcuate nucleus.\textsuperscript{41,42} Acyl ghrelin activates GHS-Rs on neuropeptide Y/agouti-related protein (NPY/AgRP) neurons in arcuate nuclei and releases NPY and AgRP to stimulate food intake, body weight gain,\textsuperscript{43–48} and diabetic hyperphagia (Figure).\textsuperscript{49,50} Chemical ablation and double knockout of NPY and AgRP attenuates ghrelin-induced increased food intake.\textsuperscript{51,52} However, a single knockout NPY mouse model features preserved the AgRP activity, which partially compensates for the decreased ghrelin-induced food intake.\textsuperscript{23} ICV administration of AgRP is a competitive antagonist of MCRs\textsuperscript{53} and acts to increase feeding.\textsuperscript{13} AgRP is also a potent antagonist of MC receptors in weight control.\textsuperscript{42,55}
2.2 α-MSH attenuates ghrelin-elicited food intake

In rats allowed to feed ad libitum, the plasma acyl ghrelin concentration is reportedly low and reaches a peak during fasting,73 followed by a rapid decrease to the nadir level after intake.49 ICV injection of ghrelin can rapidly stimulate increased food intake.79 The effect can persist for 8h59-61 but has ceased by 12 or 24h (Table 1).59,61 ICV administration of α-MSH to rats (1.0 and 2.0 nmol/rat) significantly suppresses the ghrelin-induced increased food intake 2h after injection.61,62 The suppression can persist for 8h after injection56 with an apparent dose-dependent effect (1-6 nmol/rat),72 although no effect is evident at 24h after injection (Table).71,72 α-MSH, which displays high affinity to the MC3 and MC4 receptors, can competitively activate the MC receptors with AgRP that is stimulated by ghrelin, and can partly attenuate the effect of acyl ghrelin on food intake.51,62

Table 1

<table>
<thead>
<tr>
<th>Authors</th>
<th>Species</th>
<th>Route of drug administration</th>
<th>Food intake</th>
<th>α-MSH on food intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huang et al., 2017</td>
<td>Rat α-octanoylated ghrelin</td>
<td>ICV 0.1 nmol/rat: 1, 2, 4, 8H (†), 12, 24H (−)</td>
<td>1 nmol/rat: 1, 2, 4, 8H (†), 12, 24H (−)</td>
<td></td>
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<tr>
<td>Nakazato et al., 2001</td>
<td>Rat ghrelin</td>
<td>ICV 50 pmol/rat: 2H (†)</td>
<td>2 nmol/rat: 2H (†)</td>
<td></td>
</tr>
<tr>
<td>Lucas et al., 2014</td>
<td>Rat Rb anti-α-MSH IgG</td>
<td>ICV 1 nmol/rat: 24H (−)</td>
<td>2 nmol/rat: 2H (†)</td>
<td></td>
</tr>
</tbody>
</table>

H = hours; ICV = Intracerebroventricular; α-MSH = α-melanocyte stimulating hormones.

3. EFFECTS OF α-MSH IN GHRELIN-ELICITED GASTRIC EMPTYING

3.1. Ghrelin elicits gastric emptying

Ghrelin reportedly increases gastric emptying in conscious food-deprived rats46,64 and humans.61,64,66 ICV administration of ghrelin can increase gastric motility in a dose-dependent manner.57 However, this was not apparent in totally vagotomized rats.57 Ghrelin induces orexigenic effects by means of vagal nerve and afferent activities,68 and is a very powerful gastrokinetic agent. ICV injection of Ghrelin can induce c-fos expression in the nucleus tractus solitaries and the dorsomotor nucleus of the vagus,69 and can directly stimulate the enteric neural pathway.70

The ICV injection of ghrelin also potently stimulates feeding behavior and increases gastric emptying by activating hypothalamic NPY/AgRP neurons in arcuate nuclei.70,68 However, in rats the ICV administration of NPY suppresses postprandial antral contraction71 and delays gastric emptying.71,72 No effect on gastric emptying in humans has been observed.73 These results might hint that ghrelin-NPY signaling is not the cause of acceleration of gastric emptying.46 ICV injection of AgRP can increase feeding73 through MCR4 but the influence of AgRP on gastric motility is unknown.

Central ICV49,68 or peripheral (intravenous70,74 or intraperitoneal49) administration of ghrelin can dramatically accelerate gastric emptying. Obesity and overeating are closely linked to rapid gastric emptying. On the contrary, anorexia and cachexia are related to delayed gastric emptying.75,76 Ghrelin is a strong prokinetic agent and may be the basis of a potent method to reverse postoperative gastric ileus.77

3.2. α-MSH fails to attenuate gastric emptying elicited by ghrelin

α-MSH acting in a competitive role with AgRP on MC3 and MC4 receptors does not attenuate the gastric emptying that is accelerated by central acyl ghrelin stimulation.41 These results indicate that the accelerated gastric emptying induced by the ICV injection of ghrelin is not mediated by MCRs in the brain.

4. EFFECTS OF α-MSH SMALL INTESTINAL TRANSIT ELICITED BY GHRELIN

4.1. Ghrelin elicits small intestinal transit

Ghrelin introduced by ICV injection41 and intravenous injection53,74 increases the geometric center of intestinal transit and running percentage of small intestinal transit.41 The intraperitoneal injection of ghrelin and oral administration of ghrelin receptor agonist also accelerate small intestinal transit.44 Ghrelin acts on the receptors in the intestinal neuromuscular tissue to accelerate the intestinal transit via cholinergic mechanisms.57 The acceleration of small intestinal transit can be affected upon the down-regulation of GHS-R1A in small intestinal muscle layers.77 The intraperitoneal injection of ghrelin is able to normalize the burn-induced79 and diabetic-related46 delay in intestinal transit. The intravenous injection of ghrelin can reverse postoperative gastric ileus in rats.41

4.2. α-MSH fails to attenuate ghrelin-elicited small intestinal transit

α-MSH acts on the MC4 receptor, which is highly enriched in peptide YY expressing enteroendocrine L cells, to induce the release of peptide YY.73 The intravenous administration of peptide YY inhibits intestinal transit.73,81 A study in rats reported that the ICV injection of α-MSH at a dose of 2 nmol/rat attenuated the increase in the geometric center, induced by ghrelin ICV injection, but not the running percentage in small intestinal transit.41 These results offer support for the view that central acyl ghrelin accelerates the small intestinal transit at least in part via MC receptors in the brain.

5. EFFECTS OF α-MSH IN GHRELIN-ELICITED COLONIC TRANSIT

5.1. Ghrelin elicits colonic transit

ICV injection of ghrelin can accelerate colonic transit time41,83,84 and can increase fecal pellet output.41,43 Intravenous injection of ghrelin does not have these effects.41 Intraperitoneal injection of ghrelin does not accelerate colonic transit,49 but does increase fecal output.49 Central administration of ghrelin can moderate gastrointestinal motor functions at paraventricular nuclei mediated by NPY,24 CRF, receptor-dependent mechanisms.83 Central or peripheral administration of NPY receptor antagonist can attenuate the ghrelin-induced increase of colonic transit.49,56 The effect of central acyl ghrelin on colonic motor functions through the action of AgRP on MC receptors is still uninvestigated.

5.2. α-MSH partly attenuates ghrelin-elicited colonic transit

α-MSH displays high affinity to the MC3 and MC4 receptors. It does not attenuate the accelerated colonic transit induced by ICV injection of ghrelin.41 However, α-MSH decreases the increases in fecal pellet and total fecal weight that are induced by ICV injection of ghrelin.41 These findings imply that distal colonic motility and secretion, similar to fecal pellet and total fecal weight, are partly mediated by MC receptors in the brain.
In conclusion, central-ghrelin-induced acceleration of gastric emptying is not mediated by MC4Rs, but the acceleration of the small intestinal transit at least is partly via MC4Rs in the brain. Distal colonic motility and secretion, similar to fecal pellet and total fecal weight, is partly mediated by MC4Rs in the brain. The various interplays between acyl ghrelin and MCRs may provide a new therapeutic avenue for ameliorating anorexia and constipation.

Previous Presentations: Parts of the content were presented at the Asian Pacific Digestive Week in Kobe, Japan on November 5, 2016.

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Halofuginone protects HUVECs from H$_2$O$_2$-induced injury by modulating VEGF/JNK signaling pathway

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Abstract

Background: Halofuginone, which is the main active ingredient of Dichroa fabrifluga, was used to inhibit the synthesis of type I collagen and played increasingly important roles in tumor therapy. This study aims to investigate the protective effects of halofuginone on human umbilical endothelial cells (HUVECs) from H$_2$O$_2$-induced apoptosis and oxidative stress.

Methods: Propidium iodide and Annexin-V double staining assay was used to measure the apoptosis. Cell viability assay, the measurements of reactive oxygen species (ROS) parameters malondialdehyde and superoxide dismutase, western-blot assays, and quantitative PCR were used to elucidate the effects and mechanisms of halofuginone in protecting H$_2$O$_2$-induced injury.

Results: The results showed that halofuginone counteracted H$_2$O$_2$-induced cell viability decline and PCNA downregulation. Furthermore, halofuginone decreased ROS levels and protected HUVECs from H$_2$O$_2$-induced apoptosis. In detail, it showed that H$_2$O$_2$ induced a transient activation of Mitogen-activated protein kinases members ERK1/2 and p38, whereas induced a sustained activation of c-Jun N-terminal kinase (JNK), which play dominant roles in triggering apoptosis. Inhibition of JNK activation also inhibited H$_2$O$_2$-mediated apoptosis. Finally, it was shown that halofuginone upregulated VEGF expressions, which functioned by inhibiting sustained JNK activation, thus protecting HUVECs.

Conclusion: Halofuginone has powerful effects in protecting HUVECs from H$_2$O$_2$-induced apoptosis, via upregulating VEGF and inhibiting overactivated JNK signaling. Halofuginone might be a promising preventive drug for cardiovascular diseases.

Keywords: Apoptosis; C-Jun N-terminal kinase; Halofuginone; Oxidative stress; Vascular endothelial cell

1. INTRODUCTION

Cardiovascular diseases (CVDs) have become the major cause of death and illness worldwide.1,2 Vascular endothelial cells have a series of complicated physiological function and play important role in the maintenance of vascular homeostasis.3 Among these diseases, injury of endothelial cells is the first initiating step of pathogenesis and endothelial malfunction is a major factor that contributes to CVDs.4,5 Oxidative stress has been demonstrated to play important roles in the pathogenesis of CVDs, consisting of superoxides, peroxides, and free radicals.6 Normally, reactive oxygen species (ROS) function as important signaling transducers, whereas overproduction of them lead to the malfunction of various tissues or organs.7,8 Especially in atherosclerosis, lipid peroxidation damage endothelial cells and their functions, inducing endothelial cell apoptosis, stimulating endothelial cell synthesis of platelet activating factor, causing platelet and neutrrophils aggregation and promoting inflammation, etc.7,9,10

Dichroa fabrifluga is a traditional Chinese medicine that has been used in China for hundreds of years with significant antimarial efficacy. Halofuginone (C$_{14}$H$_{17}$BrClN$_3$O$_4$) is a halogenated derivative of febrifugine, which is the main active ingredient of Dichroa fabrifluga. Halofuginone has the advantages of increasing the drug efficacy and reducing the gastrointestinal toxicity of febrifugine. Previous reports indicated that halofuginone could regulate cell growth and differentiation, apoptosis, cell migration, and immunity.11,12 Meanwhile, halofuginone has been shown to exhibit promising antioxidant effect.13 Currently, halofuginone has been studied extensively as promising drugs for antifribrosis and antitumor effects.12,14 However, little is known about the effects of halofuginone on cardiovascular system, especially on endothelial cells. This study mainly investigated the protective effects of halofuginone on H$_2$O$_2$-induced apoptosis in vascular endothelial cell and preliminarily explored its molecular mechanisms.

2. METHODS

2.1. Cell culture

Human umbilical vein endothelial cells (HUVECs) were obtained from Institute of Biochemistry and Cell Biology, CAS (Shanghai, China) and cultured in DMEM (Gibco, Thermo Fisher Scientific, Waltham, MA USA) medium containing 10% fetal bovine serum (FBS) and antibiotics at 37°C under 5% CO$_2$ environment.

2.2. Determination of cytotoxicity by MTT assay

Halofuginone was purchased from Sigma (St. Louis, MO, USA), the purity of halofuginone was ≥95.0% by HPLC. The MTT assay is based on the principle that viable cells convert MTT into an insoluble formazan salt. Briefly, HUVECs were cultured in 96-well plates over night at the density of 1 × 10$^4$ per well and were treated with indicated concentrations of halofuginone and/or H$_2$O$_2$ (0.5 mmol/l) for indicated time. The reason for using this H$_2$O$_2$ concentration was referred to previous reports.11,16 This...
2.3. Malondialdehyde and superoxide dismutase assay
HUVECs were cultured at a density of $2 \times 10^5$ per well in six-well plates and then treated for 24 hours with halofuginone (200 nmol/l) before stimulated with H$_2$O$_2$ (0.5 mmol/l) for 4 hours. Then assay kits (Jiancheng Bioengineering Institute, Nanjing, China) were used to measure the concentrations of malondialdehyde (MDA) and superoxide dismutase (SOD) in the cell lysates, according to the manufacturer’s protocols.

2.4. Intracellular ROS quantification
The levels of intracellular ROS were determined by the fluorescence probe dihydroethidium (DHE) (ThermoFisher Scientific, Waltham, MA, USA). Briefly, $2 \times 10^5$ HUVECs were cultured in six-well plates and were treated with halofuginone and/or H$_2$O$_2$ for indicated time, then cells were washed with PBS, then incubated with 10 μmol/l DHE dissolved in DMEM medium for 30 min at 37ºC. Subsequently, cells were washed with PBS twice and analyzed by Fluoroskan Ascent Fluorometer (ThermoFisher, Helsinki, Finland).

3. RESULTS

3.1. Halofuginone protected HUVECs from H$_2$O$_2$-induced injury
The chemical structure of halofuginone was shown in Figure 1A. To show the extent of lipid peroxidation on cell membrane, cell viability, which was measured using the MTT assay, was used, which could reflect the levels of cell proliferation and viability. It was demonstrated that H$_2$O$_2$ induced a significant decrease of cellular viability, whereas halofuginone intervention significantly attenuated H$_2$O$_2$-induced decrease of cellular viability (Figure 1C, p < 0.001). Moreover, the expression levels of another parameter, PCNA, was used, which could also reflect the levels of cell proliferation and viability. It was demonstrated that H$_2$O$_2$ induced a significant decrease of PCNA expression level, whereas halofuginone treatment partially attenuated H$_2$O$_2$-mediated down regulation of PCNA (Figure 1D). These findings suggested that halofuginone protected HUVECs from H$_2$O$_2$-induced injury.

3.2. Halofuginone protected HUVECs by counteracting H$_2$O$_2$-induced apoptosis
Then, we investigated the mechanisms of halofuginone-mediated protection of HUVECs. At first, we measured the ROS levels and corresponding parameters of ROS. MDA is an index of lipid peroxidation, whilst SOD is an antioxidant enzyme to prevent injuries from ROS. Halofuginone protected HUVECs. At first, we measured the ROS levels indicated by DHE. Meanwhile, halofuginone protected HUVECs by decreasing the levels of MDA and upregulating the levels of SOD (Figure 2A–C). Furthermore, we measured the apoptosis rates by flow cytometry of HUVECs upon these treatments. It showed in Figure 2D that H$_2$O$_2$ treatment induced a significant increase of apoptosis rates, which meant increased cell injury and cell death of HUVECs, while halofuginone intervention reduced the apoptosis rates, indicating protection against ROS-induced injury. In addition, we detected the expression levels of several important proteins in apoptosis pathway by Western blotting. It showed in Figure 2E that H$_2$O$_2$ treatment induced upregulations of proapoptotic cleaved Caspase 3 and Bax, while induced downregulation of anti-apoptotic Bcl-2 protein.

3.3. Halofuginone protected HUVECs by inhibiting JNK overactivation-induced apoptosis by H$_2$O$_2$
Next, we determined the detailed mechanisms of halofuginone-protected HUVECs. It has been shown that JNK overactivation-induced apoptosis plays important roles in the maintenance of cellular functions, especially in cell survival and death. It has been shown in Figure 3A that H$_2$O$_2$ treatment caused increased phosphorylations of extracellular regulated kinase 1/2 (ERK1/2), p38, and c-Jun N-terminal kinase (JNK) in a relative short time (0.5 and 2 hours), while at the time of 24-hour treatment, the phosphorylations of JNK increased and ERK1/2 and p38 decreased. Meanwhile, a ROS scavenger, NAC could attenuate the activation of all these kinases.

2.7. Quantitative real-time PCR
The mRNA levels of human VEGF in HUVECs were detected using SYBR Green Mix (Bio-Rad, Hercules, CA, USA) and an iQ5MTM system (Bio-Rad). The experiment has been repeated for three times and been normalized to GAPDH mRNA. The primers used were as follows: VEGF: Forward (5´-ACTGGACCCCTGCTTACTGCT-3´) and Reverse (5´-TGATCCCGCAGATCTGCGTTG-3´) and GAPDH: Forward (5´-AAGCCCGGGCCACTTGA-3´) and Reverse (5´-GACCTGTTGCATGAGCCTTTCA-3´).

2.8. Statistical analysis
All data were normalized to control values of each assay and were presented as mean ± standard error of mean (SEM). Data were analyzed by one-way ANOVA followed by a Bonferroni’s post hoc tests by Graphpad Prism 6 (La Jolla, CA, USA). A two-sided p < 0.05 was considered as statistically significant.
Fig. 1 Effects of halofuginone treatment on the cell viability of HUVECs. A, The chemical structure of halofuginone and corresponding molecular formula. B, Cell viability assay of HUVECs treated with different concentrations of halofuginone (0, 50, 100 and 200 nmol/l). The optical density values were detected after 48 hours of treatment. C, Cell viability of HUVECs treatment with 0.5 mmol/l H$_2$O$_2$ and 200 nmol/l HF plus H$_2$O$_2$ for 24 hours. Cell viability was quantified by MTT assay; ***p < 0.001. D, The expression levels of PCNA upon treatments with H$_2$O$_2$ and HF plus H$_2$O$_2$ for 24 hours. Three Western blots were quantified by Image J software and were analyzed in the right panel; ***p < 0.001. The results were expressed as the mean ± SEM of three independent experiments.

Fig. 2 Halofuginone protected HUVECs by counteracting H$_2$O$_2$-induced apoptosis. A–C, Cells were treated with or without 200 nmol/l Halofuginone for 24 hours, then stimulated with 0.5 mmol/l H$_2$O$_2$ for 4 hours. The relative levels of ROS, the concentrations of MDA and SOD of cell lysates were detected; *p < 0.05, **p < 0.01, ***p < 0.001. D, The cells were treated as [A], then the apoptosis rate of HUVECs were detected by flow cytometry; **p < 0.01, ***p < 0.001. E, The cells were treated as [D], then the cleaved-caspase 3, Bcl-2, and Bax were detected by Western blot with β-actin as the loading control. The right panels were the quantitative analysis with three replicates. Data are expressed as means ± SEM from three independent experiments; *p < 0.05, **p < 0.01, ***p < 0.001.
MAPKs (Figure 3A). This result showed a persistent activation of JNK and transient activation of ERK and p38, indicating that over-activation of JNK might play a role in the damage caused by H2O2. Therefore, using JNK specific inhibitor, SP600125, we found that inhibition of JNK activation could partially reverse the cellular injury by H2O2, indicated by cell viability assay and apoptosis counting (Figure 3B, C). Meanwhile, the treatment of SP600125 also partially attenuated the decreased expression levels of PCNA, induced by H2O2 (Figure 3D). More importantly, we investigated whether halofuginone would inhibit the overactivation of JNK, since it could suppress the ROS levels. It showed in Figure 3E that halofuginone treatment could significantly inhibit the overactivated JNK, while activated ERK1/2 and had little effects on p38 activation. These results indicated that overactivated JNK played dominant roles in initiating apoptosis, induced by H2O2, while halofuginone protected endothelial cells by inhibiting JNK phosphorylation.

### 3.4. Halofuginone-mediated VEGF upregulation exerted protective roles against H2O2 in HUVECs

Vascular endothelial growth factor (VEGF) is an important growth factor that confers protective effects on endothelial cells by activating the VEGF receptors-related signaling pathways.22 In Figure 4A, B, it was shown that halofuginone intervention upregulated both the mRNA and protein levels of VEGF. To test whether halofuginone exerted protective effects by or partially by upregulating VEGF, we used VEGF to test whether it would protect HUVECs from cell injury. It showed in Figure 4C and Figure 4D that addition of VEGF could significantly attenuate H2O2-induced cell viability decrease and apoptosis increase. Moreover, it showed that VEGF could also partially reverse the down-regulation of PCNA and the upregulation of cleaved caspase 3 by H2O2 and inhibited the overactivated JNK phosphorylation (Figure 4E). More importantly, to verify whether halofuginone exerted protective effects by or partially by
upregulation of VEGF, we used VEGF specific inhibitor, Cediranib (AZD2171). It showed in Figure 4F, G that addition of VEGF inhibitor significantly decreased the protective effects of halofuginone, indicated by cell viability assay and apoptosis counts. Meanwhile, addition of VEGF inhibitor partially blunted the antiapoptotic effects of halofuginone, indicated by increased expression of cleaved caspase 3, decreased expression of PCNA, compared with halofuginone treatment alone (Figure 4H, I). These results further verified that halofuginone protected endothelial cells by or partially by upregulating VEGF.

4. DISCUSSION
CVD is a group of chronic lipid-driven inflammatory diseases characterized by accumulation of oxidized lipids in arterial walls, which can lead to a heart attack or stroke. Endothelial cells are the first to be injured during the pathogenesis of atherosclerosis. Therefore, protection of endothelial cells from oxidative stress is very important. In our study, we showed that halofuginone protected HUVECs from H2O2-induced apoptosis. Meanwhile, it showed that overactivation of JNK played dominant roles in ROS-mediated apoptosis and halofuginone conferred protective effects by inhibiting JNK activation, while had little effects on ERK and p38 phosphorylation. Moreover,
we found that halofuginone-mediated VEGF upregulation is or partially responsible for the protective effects upon H₂O₂ intervention. Our study elucidated the protective effects and corresponding mechanisms of halofuginone on endothelial cells, suggesting multiple biologic actions of halofuginone might be a potent antagonist for ROS-induced damage.

Currently, ROS has been shown to play important roles in both physiological and pathophysiological conditions. ROS mainly consists of superoxide (O₂⁻), peroxides (H₂O₂ and ROOH), and free radicals (HO·and RO·). Overproduction of ROS has been shown to play important roles in the pathogenesis of multiple diseases. Therefore, extinguishing ROS has been used widely in the preclinical and clinical settings. In our study, we demonstrated that halofuginone effectively reduced MDA levels, a biomarker of oxidative stress, while simultaneously increasing the activity of SOD, an antioxidant enzyme. MDA levels could reflect the severity of attack in cells by free radicals, and SOD activity levels reflect the capability of scavenging oxygen free radicals. Therefore, these findings suggest that halofuginone protects HUVECs by preventing oxidative stress. In addition to increasing antioxidant activity, halofuginone has been shown to function in antitumor, antiinflammation, and antibiosis effects. Recently, halofuginone has been shown to stimulate adaptive remodeling of the vessel and enhance halofuginone treatment in balloon-injured carotid arteries. However, the mechanism is not clear. In our study, we clearly demonstrated the effects and mechanisms of halofuginone in protecting endothelial cell from ROS-mediated injuries.

Apoptosis, a form of programmed cell death, is directly or indirectly regulated by complicated pathways in the cells. Apoptosis plays an important role in tissue remodeling, aging, and immune response, while irreversible damage and abnormal apoptosis may be the cause of many diseases. Apoptosis is tightly regulated in the cells. The canonical pathway that triggers apoptosis is the Bcl-2-Bax/Bak pathway. The antiapoptotic protein Bcl-2 is found located at both the cytoplasm and the mitochondria and protects cells from apoptosis by binding to the proapoptotic Bax, Bak, and the BH3-only proteins. Bcl-2 was downregulated or if it binds with BH3-only proteins, Bax and Bak will experience oligomerization and damage mitochondria, therefore triggering apoptosis. In our study, we showed that H₂O₂ treatment downregulated Bcl-2 protein and upregulated Bax protein, while halofuginone intervention reversed the downregulation of Bcl-2. Furthermore, it has been reported that Bcl-2 was regulated by multiple signaling pathways, and Bcl-2 was considered as the key protein in controlling cell fate, since it is the center of cell death, autophagy, and oxidative stress. The reasons for the changes of Bcl-2 may attribute to the changes of JNK activation, because sustained JNK activation will lead to Bcl-2 phosphorylation and changes in the conformation, releasing the proapoptotic proteins, such as Bim, Bax, Bak, etc. However, this has not further been proved in our experiments.

MAPKs is a group of kinases that involved in a variety of intracellular information transfer processes, which can react to a wide range of extracellular stimuli. They consists of ERK1/2, p38, and JNK kinases and are mainly involved in cellular inflammatory response and apoptosis under the condition of stress. In our study, it was demonstrated that H₂O₂ induced a transient activation of ERK1/2, p38, and JNK in a short time <2 hours, which might be a stress response of cells. However, at 24 hours JNK experienced a persistent activation. JNK, as an important number of MAPKs, induces multiple biological events and regulates cell death and survival upon cell stimuli. It has also been proved that transient JNK activation is related to cell survival, whereas prolonged JNK activation is associated with apoptotic cell death. Other studies also indicated that transiently activated JNK triggers Bcl-2 phosphorylation at several amino acid residues, which increases cell survival via disruption of the interaction of Beclin1 and Bcl-2. However, prolonged JNK activation promoted the release of cytochrome C and the cleavage of caspase-3, which results in apoptosis. It was shown in our study that H₂O₂ induced a persistent JNK activation, inhibition of JNK by SP600125 could significantly attenuate H₂O₂-mediated apoptosis. Furthermore, halofuginone-mediated VEGF upregulation also inhibited JNK phosphorylation. Therefore, inhibition of JNK by halofuginone might be the key mechanism of protection against H₂O₂ injuries.

VEGFs and their receptors (VEGFRs) have emerged as the principal drivers of angiogenesis and lymph-angiogenesis, and hence the development and maintenance of both of these vascular systems. Now, VEGF/VEGFR signaling have been considered essential in the pathogenesis of CVD. Activation of VEGFR-2 by VEGF leads to stimulation of various intracellular signaling cascades, including activation of the ERK1/2 and p38 MAPK pathways, which mainly confer protective effects on endothelial cells. In our study, we showed that VEGF supplementation decreased H₂O₂-induced apoptosis and inhibited overactivated JNK. Meanwhile, halofuginone treatment activated ERK1/2, which might correlate with VEGF upregulation and conferred protective effects. However, in our study, we did not further investigate the mechanism of VEGF upregulation upon halofuginone. These results demonstrate that halofuginone protects HUVECs from apoptosis and elucidate a new pathway.

In conclusion, halofuginone has powerful effects in protecting HUVECs from H₂O₂-induced apoptosis, via upregulating VEGF and inhibiting overactivated JNK phosphorylation. These findings suggest that halofuginone might be a potent antioxidant agent and promising preventive drug for CVDs. Further studies will be necessary to determine the exact effects of halofuginone on cardiovascular disease.

REFERENCES


Elevated serum ferritin level associated with hepatic steatosis and fibrosis in hepatitis C virus–infected patients

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Abstract

Background: Serum ferritin is an indicator of iron accumulation in a human body, and it is frequently elevated in patients with systemic inflammatory state in chronic hepatitis C (CHC). Iron accumulation is associated with hepatic fibrosis, steatosis, and unfavorable outcome in CHC patients. We studied the status of elevated serum ferritin level and its association with the liver fibrosis or steatosis in Taiwanese CHC patients.

Methods: Seven hundred and thirty-eight Taiwanese CHC patients were consecutively included in this study. Laboratory analysis, four indexes of fibrosis (FIB4), histological assessment of fibrosis, and steatosis were assessed by appropriate elevation of serum ferritin level.

Results: Three hundred and one patients (40.8%) had elevated serum ferritin level (sex-specific threshold >1.5 × upper limit of normal). Serum iron level (odds ratio [OR], 1.02; 95% CI, 1.01%-1.03%; p = 0.001), female gender (OR, 1.49; 95% CI, 1.07%-2.08%; p = 0.018), serum gamma-glutamyl transferase level (OR, 1.07; 95% CI, 1.03%-1.10%; p = 0.001), steatosis grade (OR, 1.56; 95% CI, 1.13%-2.16%; p = 0.006), and FIB4 ≥3.25 (OR, 1.63; 95% CI, 1.18%-2.27%; p = 0.003) indexes were associated with high serum ferritin level by multivariate logistic regression analysis. Patients with steatosis (>5%) were associated with older age (OR, 1.01; 95% CI, 1.00%-1.03%; p = 0.015), body mass index (OR, 1.10; 95% CI, 1.05%-1.15%; p < 0.001), and elevated serum ferritin level (OR, 1.01; 95% CI, 1.00%-1.01%; p = 0.024) by multivariate logistic regression analysis. Serum ferritin level also associated with high FIB4 (≥3.25) (OR, 1.01; 95% CI, 1.00%-1.002%; p = 0.010) when multivariate model adjusted together with advanced liver fibrosis by biopsy.

Conclusion: Elevated serum ferritin level was noted in 40.8% of Taiwanese CHC patients, and the serum ferritin level was associated with liver steatosis and high FIB4.

Keywords: CHC; Ferritins; FIB4; HCV; Liver cirrhosis

1. INTRODUCTION

Globally over 170 millions of people were infected by hepatitis C virus (HCV), a prevalence of 2.8% to 3% of the World population, and it is a serious burden to global health.1 Up to 20% of patients with chronic hepatitis C (CHC) would develop liver cirrhosis, and >25% of patients who had cirrhosis would develop severe liver failure or hepatocellular carcinoma and needed liver transplantation.2 Host genetic background, HCV viral load and genotype, and environmental factors are the risk for the clinical manifestation and progression of the liver failure. However, HCV interferes with the host iron metabolism, and it is related to the increased hepatic and serum iron components.3,4 An elevated serum ferritin level is associated with some chronic liver diseases such as nonalcoholic fatty liver disease, nonalcoholic steatohepatitis,4 steatosis caused by HCV, and liver fibrosis progression5 and is also related to HCV treatment outcome.6 Elevated serum ferritin level has also been previously observed in obesity-related chronic inflammatory conditions, such as diabetes and metabolic syndrome.7 Also elevated serum ferritin level can predict early mortality of patients with decompensated liver cirrhosis as a surrogate marker.8 Cutoff point of the serum ferritin level was calculated as 350 ng/mL in women and 450 ng/mL in men to predict advanced hepatic iron deposit in a report from Italy.9
Taiwanese CHC patients. However, Lin et al reported that both serum and hepatic iron depositions did not relate to grade or stage of liver histology. Therefore, we studied the status of elevated serum ferritin level and its association with the liver fibrosis or steatosis in a large number of Taiwanese CHC patients.

2. METHODS

In total, 738 Taiwanese patients who underwent a diagnostic liver biopsy before treatment at Kaohsiung Medical University Hospital, a tertiary medical center, were included in this study. All patients infected with HCV were proven seropositive for anti-HCV antibody. None of the patients included in this study were positive for hepatitis B virus and human immunodeficiency virus; we also excluded patients who drink >60 g of alcohol per day, and those with hereditary hemochromatosis and hepatocellular carcinoma.

Before the initiation of HCV treatment, general demographic characteristics and serum biochemical analyses using commercial tests were performed. These included glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), gamma-glutamyl transferase (GGT), alpha fetoprotein (AFP), and platelet counts. Biochemical tests and complete blood counts including serum ferritin and iron levels were performed using a standard autoanalyzer. The serum level of HCV-RNA was measured using RT-PCR method and Cobas Amplicor HCV test, V2.0 (Roche Diagnostics, Branchburg, NJ). For calculating body mass index (BMI), we used following formula: weight in kilogram/height in meter$^2$. Liver biopsy was performed by a single pathologist who was blind to the treatment. Liver biopsy was evaluated according to the METAVIR scoring system, and the degree of steatosis was graded in four stages (grade 0, <5%; grade 1, 5%-33%; grade 2, 34%-66%; grade 3, >66%). Serum ferritin was considered elevated if it was >350 ng/mL in women and >450 ng/mL in men. The four indexes of fibrosis (FIB4) were calculated to describe advanced fibrosis. We used following formula to calculate FIB4:

$$FIB4 = \frac{(age \ [years] \times \ GOT \ [U/L])}{(platelet \ [10^9/L] \times \sqrt{GPT \ [U/L]})}.$$  

Aspartate aminotransferase to platelet ratio index (APRI) was calculated by the following formula:

$$\text{APRI} = \frac{\text{GOT} \ [U/L] / \text{GOT \ [upper limit of normal range]}}{\text{Platelet} \ [10^9/L]}.$$  

2.1. Statistical analysis

Analyzing the relation between serum ferritin level and other variables of interest, we defined sex-specific serum ferritin level as a dichotomous variable with 350 ng/mL in women and 450 ng/mL in men, a 1.5-fold increased value of normal ferritin level in serum. Descriptive statistics were applied for data distribution, mean, and standard deviation. Group means were compared using analyses of variance and Students $t$ test for parametric or nonparametric test. For association between baseline predictors of both serum ferritin level and hepatic steatosis score, we used a multiple logistic, linear regression, and Fisher’s exact or chi-square tests were performed when appropriate. All statistical analyses were performed using the IBM SPSS Statistics, version 20 and original patient’s data gathered in Microsoft Excel software. All statistical analyses were based on two-sided hypothesis tests with a significance level of $p < 0.05$.

3. RESULTS

3.1. Associated factors for sex-specific high ferritin level

All patients were separated into two groups by sex-specific, 1.5-fold increased serum ferritin level, and the basic characteristics of 738 patients are summarized in Table 1. The percentage of female patients with 1.5-fold high serum ferritin level was higher compared with the percentage of those with lower serum ferritin level (48.8% vs 40.5%; $p = 0.025$). An elevated ferritin level was associated with older age (55.3 ± 9.2 vs 52.0 ± 11.2; $p = 0.0001$), the presence of diabetes (21.4% vs 13.9%; $p = 0.008$), the presence of steatosis (13.3% vs 5.9%; $p = 0.001$), and the stage of fibrosis ($p = 0.044$). Therefore, BMI, HCV genotype, and viral load were not so important for high ferritin level. However, serum biochemical parameters were associated with sex-specific high ferritin level and simple, noninvasive predictor

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic comparison between patients with and without increase of ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
<td>Total, N = 738</td>
</tr>
<tr>
<td>Sex, N (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>414 (56.1)</td>
</tr>
<tr>
<td>Female</td>
<td>324 (43.9)</td>
</tr>
<tr>
<td>Age, mean ± SD, y</td>
<td>53.3 ± 10.6</td>
</tr>
<tr>
<td>BMI, mean ± SD, kg/m²</td>
<td>25.0 ± 3.4</td>
</tr>
<tr>
<td>HCV type, n (%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>373 (50.5)</td>
</tr>
<tr>
<td>Other</td>
<td>365 (49.5)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>123 (17)</td>
</tr>
<tr>
<td>No</td>
<td>600 (83)</td>
</tr>
<tr>
<td>HCV-RNA, log IU/mL, mean ± SD</td>
<td>5.4 ± 2.1</td>
</tr>
<tr>
<td>Steatosis, n (%)</td>
<td></td>
</tr>
<tr>
<td>&gt;34%</td>
<td>66 (9.0)</td>
</tr>
<tr>
<td>&lt;33%</td>
<td>672 (91.1)</td>
</tr>
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<td>Fibrosis grade, n (%)</td>
<td></td>
</tr>
<tr>
<td>F0</td>
<td>78 (10.6)</td>
</tr>
<tr>
<td>F1</td>
<td>220 (29.8)</td>
</tr>
<tr>
<td>F2</td>
<td>223 (30.2)</td>
</tr>
<tr>
<td>F3</td>
<td>119 (16.1)</td>
</tr>
<tr>
<td>F4</td>
<td>98 (13.3)</td>
</tr>
</tbody>
</table>

BMI = body mass index; HCV = hepatitis C virus; ULN = upper limit of normal.
Table 2
Comparison of laboratory parameters in sex-specific 1.5-fold increase of ferritin

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total, N=738</th>
<th>&lt;1.5×ULN, ng/mL, N=437</th>
<th>≥1.5×ULN, ng/mL, N=301</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Value</td>
<td>N</td>
</tr>
<tr>
<td>GOT, U/L</td>
<td>106.2 ± 55.3</td>
<td>437</td>
<td>99.7 ± 54.8</td>
</tr>
<tr>
<td>GPT, U/L</td>
<td>157.4 ± 83.8</td>
<td>437</td>
<td>147.7 ± 81.3</td>
</tr>
<tr>
<td>GGT, U/L</td>
<td>66.0 ± 48.1</td>
<td>437</td>
<td>58.4 ± 41.4</td>
</tr>
<tr>
<td>Platelet, 10^9/L</td>
<td>161.5 ± 86.6</td>
<td>437</td>
<td>168.1 ± 82.3</td>
</tr>
<tr>
<td>AFP, ng/mL</td>
<td>16.0 ± 24.1</td>
<td>437</td>
<td>12.6 ± 18.3</td>
</tr>
<tr>
<td>Iron, µg/dL</td>
<td>42.3 ± 19.7</td>
<td>437</td>
<td>38.7 ± 16.1</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>103.5 ± 55.6</td>
<td>320</td>
<td>99.1 ± 60.3</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>168.5 ± 33.2</td>
<td>319</td>
<td>168.1 ± 34.0</td>
</tr>
<tr>
<td>APRI</td>
<td>1.7 ± 1.4</td>
<td>437</td>
<td>1.6 ± 1.3</td>
</tr>
<tr>
<td>FIB4</td>
<td>3.5 ± 2.9</td>
<td>437</td>
<td>3.3 ± 3.0</td>
</tr>
</tbody>
</table>

Results in mean ± standard deviation. AFP = alpha fetoprotein; APRI = aspartate aminotransferase to platelet ratio index; FIB4 = four indexes of fibrosis; GGT = gamma-glutamyl transferase; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; ULN = upper limit of normal.

Table 3
Multivariate analysis of associated factors for the sex-specific increase of serum ferritin

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender</td>
<td>1.49</td>
<td>1.07-2.08</td>
<td>0.018</td>
</tr>
<tr>
<td>GGT</td>
<td>1.007</td>
<td>1.003-1.011</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Iron</td>
<td>1.02</td>
<td>1.01-1.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Steatosis score (&gt;5%)</td>
<td>1.56</td>
<td>1.13-2.16</td>
<td>0.006</td>
</tr>
<tr>
<td>FIB4 (&gt;3.25)</td>
<td>1.63</td>
<td>1.18-2.27</td>
<td>0.000</td>
</tr>
<tr>
<td>BMI</td>
<td>1.02</td>
<td>0.97-1.06</td>
<td>0.400</td>
</tr>
</tbody>
</table>

BMI = body mass index; FIB4 = four indexes of fibrosis; GGT = gamma-glutamyl transferase.

4. DISCUSSION

In this study, we show that serum ferritin was independently associated with the presence of steatosis and high FIB4 score. We adopted a cutoff point of 350 ng/mL in women and 450 ng/mL in men to calculate the association from the study by Sebastiani et al. The sex-specific cutoff for serum ferritin may be useful to predict liver steatosis and fibrosis, but additional studies are needed for validation.

In our study, high serum ferritin levels were strongly associated with steatosis and fibrosis in CHC patients. Also Vagu et al. reported that an elevated serum ferritin level can represent an early marker for the severity of chronic liver disease, related to the degree of liver steatosis grade. In multivariate model, these associations remained strongly significant. However, Rubbia-Brandt et al. described that HCV genotype 3 is important for liver steatosis, but HCV genotype 3 is rare in Taiwan. In our study, according to subanalyses, the association between serum ferritin and steatosis remained significant in both univariate and multivariate analyses in patients with HCV genotypes 1 and 2.

We focused on investigating the possible role of serum iron and ferritin in the development of liver steatosis. The mechanism of steatosis in HCV infection remains uncertain, which is considered to include multifunctional iron overload and insulin resistance (IR). Bugianesi et al. studied that the IR has an association with both serum ferritin level and steatosis grade. However, our study generally supports that elevated serum ferritin level occurs in type 2 diabetes, and diabetes is one of the main risk factors of increased ferritin level in HCV-infected patients by univariate analysis. In other hand, HCV may induce IR by itself in disease progression and genotype specific way.

In CHC, serum ferritin can be elevated because of HCV-induced downregulation of hepcidin. Liu et al. proved that HCV can inhibit hepcidin mRNA in Huh7.5 cell line followed by increased hepatic iron. Accumulated iron can lead to oxidative stress, hepatic fibrosis, and cirrhosis. They also reported that hepcidin reduced HCV replication in Huh7.5 cell line. Increased iron can influence the HCV replication but is more likely to contribute to disease by potentiating oxidative stress,
which leads to chronic inflammation. In most studies, HCV viral load does not correlate with disease. Sumida et al. described that there was a significant strong relations does exist between hepatic fibrosis and steatosis analyzed by linear modeling. The mechanism of this theory is steatosis that has positive correlation in lipid peroxidation and hepatic fibrosis. In fact, the elevation of marker of oxidative stress, serum thioredoxin, has an association with hepatic fibrosis and the serum lipid peroxide level in HCV-infected patients. In our study, there was significant correlation between liver steatosis and hepatic fibrosis grade in univariate analysis but not in multivariate logistic regression analysis.

Steatosis and elevated serum ferritin levels were associated with elevated GGT level as a result of lipid peroxidation and development of hepatocellular carcinoma. Serum GGT level is considered a marker of severe liver diseases in CHC and liver fat deposition. An elevated serum ferritin level served to predict the sign of hepatic steatosis in nonalcoholic fatty liver disease, and also elevated serum GGT level is considered to be associated with liver steatosis in HCV-infected patients.

Fibrosis, steatosis, and serum ferritin are important in CHC progression. Interaction between fibrosis, steatosis, and serum ferritin in pathogenesis of CHC still remains uncertain. FIB4 is an accurate noninvasive marker to predict liver fibrosis. This is the first study to investigate the relations between serum ferritin and liver fibrosis by using FIB4 score. Serum ferritin level was significantly associated with high FIB4 score in univariate and multivariate analyses.

This study has few strengths and limitations. The strengths include the large sample size and all patients with the liver biopsy to diagnose steatosis and available data to calculate FIB4. Therefore, the multivariate regression analysis was accurately calculated to evaluate association between serum ferritin and moderate steatosis, advanced fibrosis by FIB4. On the other hand, there were limitations, including we did not consider about hereditary hemochromatosis and HFE gene mutations played minor role in elevating serum ferritin or iron in Taiwanese CHC patients. It is also unclear that our findings may be extrapolated to the patients for other ethnicities; however, in this study we included Taiwanese patients.

### Table 4

Univariate analysis of associated factors with the steatosis <5% vs >5%

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total, N = 738</th>
<th>Steatosis 0%-5%, N = 376</th>
<th>Steatosis &gt;5%, N = 362</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>414 (56.1)</td>
<td>219 (56.2)</td>
<td>195 (53.9)</td>
<td>0.231</td>
</tr>
<tr>
<td>Female</td>
<td>324 (43.9)</td>
<td>157 (41.8)</td>
<td>167 (46.1)</td>
<td></td>
</tr>
<tr>
<td>Age, mean ± SD, y</td>
<td>53.3 ± 10.6</td>
<td>52.3 ± 10.2</td>
<td>54.4 ± 10.9</td>
<td>0.007</td>
</tr>
<tr>
<td>BMI, mean ± SD, kg/m²</td>
<td>25.0 ± 3.4</td>
<td>24.4 ± 3.4</td>
<td>25.6 ± 3.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>HCV type, n (%)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>373 (50.5)</td>
<td>195 (51.9)</td>
<td>178 (49.2)</td>
<td>0.465</td>
</tr>
<tr>
<td>Other</td>
<td>365 (49.5)</td>
<td>181 (48.1)</td>
<td>184 (50.8)</td>
<td></td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>123 (17)</td>
<td>55 (14.8)</td>
<td>68 (19.3)</td>
<td>0.108</td>
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<tr>
<td>No</td>
<td>600 (83)</td>
<td>316 (85.2)</td>
<td>284 (80.7)</td>
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<tr>
<td>HCV-RNA, log IU/mL, mean ± SD</td>
<td>5.4 ± 2.1</td>
<td>5.3 ± 2.2</td>
<td>5.4 ± 2.1</td>
<td>0.691</td>
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<tr>
<td>Fibrosis grade</td>
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<td></td>
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<tr>
<td>F0-F2</td>
<td>521 (70.6)</td>
<td>282 (75)</td>
<td>239 (68)</td>
<td>0.007</td>
</tr>
<tr>
<td>F3-F4</td>
<td>217 (29.4)</td>
<td>94 (25)</td>
<td>123 (34)</td>
<td></td>
</tr>
</tbody>
</table>

BMI = body mass index; HCV = hepatitis C virus.

### Table 5

Univariate analysis of laboratory parameters with the steatosis <5% vs >5%

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total, N = 738</th>
<th>Steatosis 0%–5%, N = 376</th>
<th>Steatosis &gt;5%, N = 362</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOT, U/L</td>
<td>106.2 ± 55.3</td>
<td>106.4 ± 58.2</td>
<td>106.1 ± 52.3</td>
<td>0.943</td>
</tr>
<tr>
<td>GPT, U/L</td>
<td>157.4 ± 83.8</td>
<td>157.6 ± 87.2</td>
<td>158.1 ± 80.2</td>
<td>0.827</td>
</tr>
<tr>
<td>GGT, U/L</td>
<td>66.0 ± 48.1</td>
<td>60.0 ± 44.9</td>
<td>62.3 ± 50.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Platelet, 10⁹/L</td>
<td>161.5 ± 58.6</td>
<td>163.3 ± 59.5</td>
<td>159.6 ± 57.6</td>
<td>0.395</td>
</tr>
<tr>
<td>APF, ng/mL</td>
<td>16.0 ± 24.1</td>
<td>15.2 ± 27.1</td>
<td>17.0 ± 20.6</td>
<td>0.319</td>
</tr>
<tr>
<td>Ferritin, ng/mL</td>
<td>392.6 ± 245.2</td>
<td>388.1 ± 237.1</td>
<td>418.0 ± 251.1</td>
<td>0.006</td>
</tr>
<tr>
<td>Iron, µg/dL</td>
<td>42.3 ± 19.7</td>
<td>43.5 ± 20.9</td>
<td>41.0 ± 18.4</td>
<td>0.092</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>103.5 ± 55.6</td>
<td>95.3 ± 41.8</td>
<td>110.7 ± 64.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>168.5 ± 33.2</td>
<td>166.9 ± 35.5</td>
<td>169.8 ± 31.1</td>
<td>0.319</td>
</tr>
</tbody>
</table>

Results in mean ± standard deviation.

AFP = alpha fetoprotein; GGT = gamma-glutamyl transferase; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase.

### Table 6

Multivariate analysis for the associated factors for steatosis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.01</td>
<td>1.00-1.03</td>
<td>0.015</td>
</tr>
<tr>
<td>Advanced fibrosis</td>
<td>1.29</td>
<td>0.92-1.90</td>
<td>0.135</td>
</tr>
<tr>
<td>Ferritin</td>
<td>1.001</td>
<td>1.00-1.001</td>
<td>0.024</td>
</tr>
<tr>
<td>BMI</td>
<td>1.10</td>
<td>1.05-1.15</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

BMI = body mass index.
In conclusion, we show that serum ferritin is strongly associated with the presence of steatosis in liver and high noninvasive fibrosis marker FIB4. The sex-specific cutoff for serum ferritin maybe used to evaluate the steatosis in clinic, but further investigation is needed.

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Retrospective analysis of endoscopic management of foreign bodies in the upper gastrointestinal tract of adults

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Abstract

Background: Foreign body impaction in the upper gastrointestinal (UGI) tract is considered an emergency worldwide. This article reports our experience in the endoscopic management of foreign bodies in the UGI tract of adults.

Methods: A retrospective chart review was conducted on adult patients (aged >18 years) who received endoscopic management of foreign bodies in the UGI tract at Shuang Ho Hospital between November 2008 and November 2016.

Results: A total of 280 patients (male/female: 107/178; mean age: 56 years) were included. Fish bones were the most common ingested foreign bodies (n = 162; 56.8%), and the esophagus was the most common lodgment site (n = 222; 77.9%). The presence of symptoms indicated that the ingested foreign bodies were lodged in the hypopharynx or esophagus rather than in the stomach or duodenum (p < 0.01). The detection rate of ingested foreign bodies in the UGI tract through plain radiography was 53% (122/230). The average “door-to-scope” was 5.9 hours, and 99.2% of the patients received endoscopic management of the ingested foreign bodies within 24 hours. The complication rate was relatively low (n = 14; 4.9%). No patient received surgical intervention or died of endoscopic management.

Conclusion: Endoscopic management is a safe and highly effective procedure for extracting ingested foreign bodies. Rapid endoscopic intervention should be provided to reduce the risk of complications.

Keywords: Esophagus; Foreign bodies; Gastrointestinal tract; Hypopharynx

1. INTRODUCTION

Ingestion of foreign bodies is a relatively common clinical problem in emergency departments worldwide. Most ingested foreign bodies (80%-90%) pass spontaneously. However, approximately 10% to 20% of foreign bodies require an endoscopic procedure for removal and <1% require surgery. Approximately 1500 deaths occur in the United States annually because of ingestion of foreign bodies. Foreign body ingestion is associated with a high risk of complications because of the size or shape of the foreign body or the host’s comorbidity. Foreign body ingestion occurs most commonly in children (80%), with a peak incidence from 6 months to 3 years of age. The remaining patients (20%) are adults. By contrast, adult patients with mental or psychiatric disorders, alcoholism, and drug abuse may ingest foreign bodies with nonfood objects. Foreign body ingestion and food bolus impaction are extremely common in Taiwan. Because only a few cases of foreign body ingestion have been reported in Taiwan, the aim of this study was to report our experiences in the endoscopic management of foreign bodies in the upper gastrointestinal (UGI) tract.

2. METHODS

2.1. Patients

A retrospective chart review was conducted on adult patients (aged >18 years) who received endoscopic management of foreign bodies in the UGI tract at Shuang Ho Hospital between November 2008 and November 2016. In total, 280 patients (285 incidents) who met the inclusion criteria were enrolled in the study.

2.2. Endoscopic procedures and settings

In this study, most of the patients were initially screened by an otorhinolaryngologist and examined through either plain radiography or computed tomography (CT). Subsequently, we used a flexible endoscope (GIF-H260; Olympus Optical Co., Ltd., Tokyo, Japan) for examination. Various endoscopic devices, including biopsy forceps, graspers, retrieval baskets, Roth nets, polypectomy snare, and overtubes, were used to remove the ingested foreign bodies, depending on their nature and location. All the patients received endoscopic management of foreign bodies under local pharyngeal anesthesia.
2.3. Data collection
In this study, clinical variables, such as age, sex, type and location of foreign bodies, symptoms, underlying gastrointestinal diseases, endoscopic methods and devices used, and complications, were analyzed. The “door-to-scope” was defined as the time interval between the patients presenting at our hospital and the endoscopy procedure being performed on the patients. Complication was defined as any adverse event, such as perforation or bleeding, which was related to foreign body injury or endoscopic procedure during manipulation of foreign body.

2.4. Statistical analysis
Categorical variables were compared using Pearson’s χ² test. A two-tailed p value of 0.05 indicated a significant difference.

3. RESULTS

3.1. Patient characteristics and clinical presentations
The mean (±SD) age of the 280 adult patients (285 incidents) who received endoscopic management of foreign bodies was 56 (±16) years. Female patients predominated in our study (n = 178; 62.5%), and 83.9% patients (n = 239) were asymptomatic. The common clinical symptoms after mis-swallowing were foreign body sensation, dysphagia, and odynophagia. Because 16.1% (n = 46) patients did not complain of any symptoms after foreign body ingestion, they were classified as asymptomatic patients. Among these patients, 44.7% (17/38) of the asymptomatic patients were classified as asymptomatic compared with the foreign bodies lodged in the hypopharynx or esophagus (11.7%; 29/247).

<table>
<thead>
<tr>
<th>Location</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypopharynx</td>
<td>25 (8.8)</td>
</tr>
<tr>
<td>Esophagus</td>
<td>222 (77.9)</td>
</tr>
<tr>
<td>U3 (≥25 cm from the incisors)</td>
<td>170 (59.6)</td>
</tr>
<tr>
<td>M3 (≥25 cm or &lt;35 cm from the incisors)</td>
<td>35 (12.3)</td>
</tr>
<tr>
<td>L3 (&lt;35 cm from the incisors)</td>
<td>17 (6.0)</td>
</tr>
<tr>
<td>Stomach</td>
<td>35 (12.3)</td>
</tr>
<tr>
<td>Duodenum</td>
<td>3 (1.1)</td>
</tr>
<tr>
<td>Total</td>
<td>285 (100)</td>
</tr>
</tbody>
</table>

3.2. Types and locations of foreign bodies
The most common site of foreign body lodgment was the esophagus (n = 222; 77.9%), with the upper esophagus (n = 170; 59.6%) being the predominant site. Other lodgment sites were the stomach (n = 35; 12.3%), pharynx (n = 25; 8.8%), and duodenum (n = 3; 1.1%; Table 1). The major types of foreign bodies were fish bones (n = 162; 56.8%), followed by food boluses (n = 31; 10.9%) and dentures (n = 23; 8.8%). Other types of foreign bodies included chicken bones, duck bones, medication foil, tongue rings, nasogastric tube fragments, coins, batteries, toothpicks, metal balls, eggshells, and plastic fragments (Table 2). Of the 31 patients with food bolus impaction in the esophagus, stomach, or duodenum, 20 patients (64.5%) presented with complications of an underlying esophageal pathology, mainly esophageal stenosis secondary to previous esophageal carcinoma following surgery or stenting (n = 7) and corrosive injury (n = 5). The remaining patients had underlying disorders such as achalasia, esophageal web, diverticulum, and peptic stricture.

<table>
<thead>
<tr>
<th>Type of foreign body</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sharp-pointed objects</td>
<td>230 (80.7)</td>
</tr>
<tr>
<td>Fish bones</td>
<td>162 (56.8)</td>
</tr>
<tr>
<td>Chicken bones</td>
<td>21 (7.4)</td>
</tr>
<tr>
<td>Duck bones</td>
<td>2 (0.7)</td>
</tr>
<tr>
<td>Dentures</td>
<td>25 (8.8)</td>
</tr>
<tr>
<td>Medication foil</td>
<td>19 (6.7)</td>
</tr>
<tr>
<td>Toothpicks</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Food bolus</td>
<td>31 (10.9)</td>
</tr>
<tr>
<td>Others</td>
<td>24 (8.4)</td>
</tr>
<tr>
<td>Total</td>
<td>285 (100)</td>
</tr>
</tbody>
</table>

3.3. Detection rates of plain radiography and CT
Before undergoing esophagogastroduodenoscopy (EGD), 230 (80.7%) and 11 (3.9%) patients received plain radiography and CT scan, respectively. The detection rate of foreign bodies in the UGI tract through plain radiography was 53% (122/230). Through plain radiography, the detection rate of foreign bodies lodged in the stomach and duodenum was higher than that of foreign bodies lodged in the pharynx and esophagus (73.9% vs 50.7%; p < 0.05; Table 3). The CT detection rate of foreign bodies, included food bolus, was 100% (11/11).

<table>
<thead>
<tr>
<th>Location of foreign body detection rate through plain radiography</th>
<th>Positive plain radiography (%)</th>
<th>Negative plain radiography (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharynx and esophagus</td>
<td>105 (50.7)</td>
<td>102 (49.3)</td>
<td>207</td>
</tr>
<tr>
<td>Stomach and duodenum</td>
<td>17 (73.9)</td>
<td>6 (26.1)</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>122 (53)</td>
<td>108 (47)</td>
<td>230</td>
</tr>
</tbody>
</table>

3.4. Timing of endoscopic management
With the exception of 34 patients (for which the time interval could not be traced), the mean “door-to-scope” was 5.9 (±5.2) hours in our study. Most patients (n = 249, 99.2%) underwent urgent (within 24 hours) EGD following foreign body ingestion. Among the patients, 66.5% (167/251) received emergency (within 6 hours) EGD examination. For sharp-pointed objects in the esophagus, 62.2% of the patients (130/209) in our study received endoscopic management within 6 hours in emergency settings.

3.5. Endoscopic management methods and devices
Selection of methods for endoscopic management depends on the type and the location of the foreign body ingested. In the study, for the retrieval of sharp-pointed foreign bodies (fish bones and medication foil), biopsy forceps and graspers were most commonly used with the overtube method (Figure 1). We used polypectomy snares, Dormia baskets, or Roth nets with the overtube method for most cases of denture retrieval (Figure). For food bolus impaction, the push technique (pushing the food bolus into the stomach) was used, followed by retrieval by using Dormia baskets or Roth nets or through piecemeal extraction, if the food bolus was too large to pass through the duodenum.

3.6. Complications
The complication rate of the endoscopic management of foreign bodies was 4.9% (14/285). Among the patients with complications, nine had minor lacerations with or without bleeding and were discharged from the emergency department.
with oral antibiotics and sucralfate. Only five patients (1.8%) were admitted to the chest surgery ward and treated with intravenous antibiotics and parenteral alimentation because of esophageal microperforation with pneumomediastinum, mediastinitis, or abscess formation (Table 4). Among them, four patients received short-term intravenous antibiotics (amoxicillin/clavulanic acid or cefmetazole) treatment and were discharged within 10 days after trying diet smoothly. Only one patient had been hospitalized for more than 30 days due to underlying comorbidity. None of them received further surgical intervention.

4. DISCUSSION

Foreign body ingestion is a common global problem. In the current study, fish bones (56.8%) were the most commonly ingested foreign bodies. This observation differed from reports from Western countries. Our observation on fish bone ingestion may be because of the high consumption of seafood in Taiwan, which is an island. Food bolus impaction (10.9%) was the second most common condition and was often combined with an underlying structural abnormality (64.5%) such as esophageal web, esophageal rings, or a benign or malignant esophageal stricture.

According to our observation, most foreign bodies were lodged in the esophagus (77.9%), predominantly in the upper third. This finding is consistent with previous studies. The esophagus has four physiologically narrow sites, namely, the upper esophageal sphincter, level of the aortic arch, main stem bronchus, and lower esophageal sphincter. In our study, most foreign bodies were inadvertently lodged in the physiologically narrow sites of the esophagus without any underlying pathology.

Table 4
Admission cases and management

<table>
<thead>
<tr>
<th>No.</th>
<th>Type of foreign body</th>
<th>Complications and management</th>
<th>Hospitalization, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chicken bone</td>
<td>Esophageal microperforation with mediastinal abscess</td>
<td>CS admission, 3 days</td>
</tr>
<tr>
<td>2</td>
<td>Fish bone</td>
<td>Esophageal microperforation with regional pneumomediastinum</td>
<td>CS admission, 4 days</td>
</tr>
<tr>
<td>3</td>
<td>Fish bone</td>
<td>Esophageal microperforation with mediastinitis and abscess</td>
<td>CS admission, 38 days</td>
</tr>
<tr>
<td>4</td>
<td>Fish bone</td>
<td>Esophageal microperforation</td>
<td>CS admission, 8 days</td>
</tr>
<tr>
<td>5</td>
<td>Fish bone</td>
<td>Microabscess over retropharyngeal space</td>
<td>ENT admission, 3 days</td>
</tr>
</tbody>
</table>

Conservative therapy: Intravenous antibiotics with parenteral alimentation.
CS = chest surgery; ENT = ear nose throat (otorhinolaryngology).
The clinical presentation of foreign body ingestion is associated with the type of foreign body, lodgment location, and duration after ingestion. Because of easy access to hospitals in Taipei, most of the patients presented at the emergency department with foreign body sensation. In our study, the presence of symptoms may show that the foreign bodies were lodged in the hypopharynx or esophagus instead of the stomach or duodenum ($p < 0.01$; Table 5).

The endoscopic strategy of foreign body ingestion management varies with the ingested foreign body type, and onset of symptoms. Hypersalivation and the inability to swallow liquids indicate complete esophageal obstruction. Once foreign bodies have traversed the esophagus, most objects pass within 4 to 6 days. Objects which are >2 to 2.5cm in diameter cannot pass through the pylorus or ileocecal valve, and objects longer than 5 to 6cm cannot pass through the duodenal sweep.\(^{11}\)

Most of the patients were examined by otolaryngologists, followed by plain radiography or CT examination before undergoing EGD. The detection rate of foreign bodies through plain radiography was 53% (122/230), which was consistent with a previous report.\(^{14}\) Plain radiography assisted in detecting the presence of as well as assessing the location, size, configuration, and number of ingested foreign bodies. CT is a considerably more sensitive method for the detection of foreign bodies than plain radiography. All patients with a risk of perforation or other complications that may require surgery should undergo CT examination.\(^{13}\) In our series, 11 patients (3.9%) received a CT scan, and in all the patients (11/11; 100%), the ingested foreign bodies were detected. Three of them had food bolus impaction that were due to underlying esophageal disease.

According to the clinical guidelines of the European Society of Gastrointestinal Endoscopy (ESGE), esophageal foreign objects and food boluses affected in the esophagus should be removed within 24 hours to reduce the risk of major complications,\(^{13}\) such as perforation with or without mediastinitis, retropharyngeal abscess, and aortoesophageal fistula. Therefore, the timing of the endoscopic management after foreign body ingestion is a crucial factor influencing the outcome. In our study, the mean "door-to-scope" was 5.9 (±5.2) hours. All except two patients (99.2%) received foreign body removal within 24 hours in urgent settings. For sharp-pointed objects, batteries, and other foreign bodies causing complete obstruction of the esophagus, retrieval is recommended within 6 hours under emergency conditions.\(^{13}\) In our study, rapid endoscopic management within 6 hours might reduce the risk of complications compared with over 6 hours for the sharp-pointed objects lodged in the esophagus (6.92% vs 7.60%; $p = 0.93$; Table 6), but no statistical significance due to small case numbers.

Various endoscopic methods and equipment were used, depending on the type and location of the ingested foreign bodies. For food bolus impaction in the esophagus, the priority endoscopic method is the "push technique." This method was used on 12 patients (41.4%). Pressure was gently applied to the food bolus using the tip of the endoscope after air insufflation. However, if gentle pressure did not dislodge the bolus, then fragmentation with a snare or pulling en bloc by using a retrieval basket or Rotb net was attempted. Approximately 75% to 100% of patients with food impactions are reported to have esophageal pathology during treatment or follow-up endoscopy.\(^{11,16}\) Esophageal stricture resulting from postoperative esophageal carcinoma or stenting and corrosive injury were the most common causes in our study (20/31; 64.5%). For linear, sharp-pointed foreign bodies, such as fish bones, biopsy forceps, or graspers, were used to hold the tip and retrieve the fish bone by using an overtube to protect the airway. For blunt or irregular sharp-pointed foreign bodies, such as dentures or medication foils, we used biopsy forceps, graspers, polypectomy snares, or baskets to retrieve these objects by using the overtube method.

The mortality associated with foreign body ingestion is not accurately known.\(^{1}\) Crucial factors, including the presence of a sharp foreign body and impaction duration, might predispose patients to complications.\(^{12}\) The mean "door-to-scope" of patients who had complications was longer than the patients who had no complications in our study (7.36 ± 5.82 hours vs 5.78 ± 5.18 hours). Early endoscopic interventions within 24 hours after ingestion are associated with favorable outcomes.\(^{13}\) If the foreign body remains affected for >24 hours, the risk of a major complication increases 14-fold.\(^{17}\) The complication rate was notably low in our study (4.9%) compared with that in another study (7%).\(^{2}\) For patients with major complications, such as large laceration wound with active bleeding or macroperforation, initial esophageal hemostasis and wound closure by hemoclips were suggested. Surgical intervention was reserved for the patients who failed of conservative therapy. In our series, five admission patients with microperforation and mediastinitis were treated via conservative therapy successfully under empiric antibiotics and parenteral alimentation. None of them had further adverse events after discharge.

All our patients received endoscopic management of foreign bodies without deep sedation because of emergency settings and the prevention of aspiration during the procedure. After successful and uncomplicated endoscopic removal of ingested foreign bodies, ESGE clinical guidelines suggest that the patient may be discharged. Oral antibiotics or sucralfate may be prescribed for minor esophageal laceration incurred during the procedure.

Our study had some limitations. First, we used "door-to-scope" instead of the time interval between foreign body ingestion and endoscopic management due to unavailable data of foreign body ingestion time. We assumed and emphasized that early "door-to-scope" may influence the outcome and reduce the risk of complications (as "door-to-balloon" in ST-elevation myocardial infarction and "door-to-needle" in acute ischemic stroke). Second, due to small case numbers and relatively low complication rate, the risk factors for foreign body removal-related complications in the UGI tract need further prospective studies with larger numbers of patients to be confirmed.

In conclusion, the ingestion of foreign bodies is a common clinical problem worldwide. Various instruments can be used to remove the ingested foreign bodies. Endoscopic management is a highly effective procedure for extracting ingested foreign bodies with relatively low complication and mortality rates. Rapid endoscopic intervention should be provided to patients who have ingested foreign bodies to reduce the risk of complications.

### ACKNOWLEDGMENTS

This study was supported by Tomorrow Medical Foundation. The authors expressed their gratitude to Wallace Academic Editing for English revision.

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**Table 5**

Comparisons between symptomatic and asymptomatic patients

<table>
<thead>
<tr>
<th>Location of foreign body</th>
<th>Symptomatic, n</th>
<th>Asymptomatic, n</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharynx and esophagus</td>
<td>218</td>
<td>29</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Stomach and duodenum</td>
<td>21</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

**Table 6**

Comparisons the complications between endoscopic management within 6 hours and over 6 hours for the sharp-pointed foreign body in the esophagus

<table>
<thead>
<tr>
<th></th>
<th>Within 6 hours</th>
<th>Over 6 hours</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complications</td>
<td>9</td>
<td>6</td>
<td>0.93</td>
</tr>
<tr>
<td>No complications</td>
<td>121</td>
<td>73</td>
<td></td>
</tr>
</tbody>
</table>
REFERENCES


Identification of a homozygous BBS7 frameshift mutation in two (related) Chinese Miao families with Bardet-Biedl Syndrome

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Abstract

Background: Bardet-Biedl Syndrome (BBS) is a genetically heterogeneous autosomal recessive disorder with a wide spectrum of clinical features. To date, mutations in 21 different genes (BBS1-21) have been identified as causing isolated or complex BBS phenotypes. In this report, we present three Chinese Miao ethnic patients who were diagnosed with BBS on the basis of characteristic clinical features and investigated the exome of these patients.

Methods: To evaluate disease genes, the Agilent SureSelect system and Illumina HiSeq 2000 platform for whole exome enrichment and sequencing (WES) were used on the proband and her mother. Variants that fit a recessive model of inheritance only were compared and filtered using public databases. Variants detected by exome sequencing were validated by Sanger sequencing. A total of 981 phenotypically normal subjects were enrolled as control data set.

Results: A frameshift homozygous germline mutation in BBS7 was detected by WES and identified by Sanger sequencing in affected individuals. This mutation was predicted to result in premature termination of exon 5 (c.389_390delAC, p.Asn130ThrfsX3; RefSeq NM_176824.2) and lead to a 133 amino acid truncated protein. The inheritance patterns in the families are consistent with autosomal recessive inheritance, and no such homozygous mutation was found in the other 981 controls.

Conclusion: This mutation has not yet been described in any reported literature, and this is the first report on BBS7 mutation in Chinese Miao families with BBS phenotypes.

Keywords: Bardet-Biedl syndrome; BBS7 gene; Frameshift; Whole exome sequencing

1. INTRODUCTION

Bardet-Biedl syndrome (BBS1; OMIM 209900) was first reported by Bardet and Biedl in the 1920s. BBS is a genetically heterogeneous autosomal recessive disorder. Its phenotypes are extremely variable, including four of six major symptoms (obesity, rod-cone dystrophy, renal abnormalities, polydactyly, male hypogonadism, and learning disabilities), or three major symptoms and at least two minor symptoms (hepatic fibrosis, diabetes mellitus, neurological, speech and language deficits, behavioral symptoms and at least two minor symptoms (hepatic fibrosis, diabetes mellitus, neurological, speech and language deficits, behavioral

To date, a minimum of 21 disease-causing BBS genes (BBS1-21) have been identified in 80% of BBS patients, with the remaining 20% lacking a molecular diagnosis. Some BBS genes appear to have a greater ethnicity-specific frequency than others do. This includes BBS1 M390R and BBS10 C91LfsX5, which are the most common alleles in Northern European individuals, but not found in patients of Middle Eastern or North African descent. However, few BBS mutations have been reported in Chinese populations.

In this report, we describe three BBS patients from two related Miao families from a mountain village of Miao nationality in the Yunnan Province of China. The affected individuals’ parents all married through Huanqin, a type of traditional arranged marriage in some parts of rural China. In this tradition, a daughter from one family marries a son from another family and in “exchange,” a daughter from that family marries a son from the first family. Our observations suggest a consanguineous relationship in generation I, despite no confirmation from the family (Figure 1). In the sixth nationwide census in 2010, the Miao population accounted for approximately 0.70% of total population (http://www.stats.gov.cn/). To keep the whole genome information, Miao BBS patients’ B cells were collected and immortalized, and B lymphoblastoid cell lines were successfully established by Epstein-Barr virus transformation as described in our previous study. Whole exome enrichment and sequencing (WES) in combination with direct Sanger sequencing of candidate genes identified an AC deletion mutation in BBS7 (c.389_390delAC, p.Asn130ThrfsX3; RefSeq NM_176824.2). This frameshift mutation was predicted to lead to the truncation of BBS7 protein.
of 133 amino acids from the protein. This is the first report of BBS7 mutation in Chinese Miao families with BBS phenotypes.

2. METHODS

2.1. Subjects

This study was approved by the Ethics Review Board of the First People’s Hospital of Yunnan Province, China (2013YL061). Informed consent was obtained from the patients’ parents and from all other participants. The two related families for the presented molecular investigation were identified in a Miao village in the Yunnan Province of China. A total of 51 Miao individuals from the same Miao village (unrelated to the two BBS families), and an additional 930 individuals outside this village (including 300 Miao people, 300 Dai people, 300 Hani people, and 30 Han people), were enrolled as phenotypically normal controls. Blood samples were collected for DNA extraction and laboratory examination. Physical examination was performed. Total body photographs were taken and included the hands, feet, and any specific dysmorphic features. Ophthalmic examination, abdomen ultrasound, and urogenital system examination were also conducted.

2.2. Whole-exome enrichment, sequencing, and bioinformatic analysis

WES was performed on proband (III-10) and her mother (II-6) (The Beijing Genomics Institute, China). Qualified genomic DNA was randomly sheared by Covaris (KBioscience, Herts, UK), and

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**Fig. 1** Two Chinese Miao families with BBS and photographs of patients III-3, III-5, and III-10. A, Results of Sanger Sequencing on exon5 of BBS7. III-3, III-5, and III-10 showed a homozygous c.389_390delAC (RefSeq NM_176824.2) germline mutation in BBS7. II-6 and III-10 were evaluated by whole exome sequencing. wt/mt, heterozygous carrier of the BBS7 c.389_390delAC mutation; mt/mt, homozygous carrier of this mutation. B, Photographs of patients III-3, III-5, and III-10. Top, typical BBS facial features of affected individuals; bottom, typical polydactyly of hands and feet of affected individuals.
the mean fragment size was 150 to 200 bp; this was then followed by library preparation using Agilent SureSelect Biotinylated RNA Library “baits.” Sequencing was performed on an Illumina HiSeq 2000 (Illumina, San Diego, CA) to generate 90-bp paired-end reads following the manufacturer’s protocol. SOAPaligner/SOAP2 was used to map reads onto the reference genome (http://soap.genomics.org.cn/). Only mapped reads were used for subsequent analysis. Variants were compared and filtered using public databases, including dbSNP (v129), 1000 Genome Project (20100208 release), and eight HapMap exomes. Only recessive models of inheritance (autosomal recessive model and X-linked recessive model) were considered because of the normal phenotypes of the parents.

2.3. Sequencing

Primers for candidate genes were designed using the online version of Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Polymerase chain reaction amplification of BBS7 exon 5 was performed with primers BBS7-E5f: 5′-GGCCTTTAACATCCTCATTTCAGCT-3′ and BBS7-E5r: 5′-CTTCCTCTCCAAACCCTTCTTCTC-3′. The sequencing reactions were performed using BigDye Terminator v3.1 and a Genetic Analyzer 3130 (Applied Biosystems). The sequence data were then aligned with the BBS7 reference sequence via the NCBI online blastn tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome).

3. RESULTS

3.1. Clinical findings

Three individuals (2 females and 1 male) from two families were diagnosed with BBS on the basis of criteria established elsewhere. Symptoms included retinal dystrophy and progressive night blindness, truncal obesity, bilateral postaxial polydactyly of hands and feet (III-3: six digits for each site), bilateral postaxial polydactyly of feet and unilateral brachydactyly of the hands (III-5, III-10: six digits for each foot; III-5: six digits on right hand; III-10: six digits on left hand), learning difficulties, renal abnormality, and other clinical features (Table 1; Figure 1).

3.2. WES results and sequencing analysis

Initial filtering of WES data through the public databases and recessive models revealed a homozygous c.389_390delAC (RefSeq NM_176824.2) germline mutation in BBS7 of the proband. In the proband, 35 reads (100%) across the mutation site showed the c.389_390delAC mutation, while in the mother, 7 out of 19 reads (36.8%) across the mutation site showed the two base deletion. This mutation was identified by Sanger sequencing, and the results revealed that the affected cousins of the proband carried this homozygous BBS7 defect as well, while their parents and some siblings were heterozygous carriers of the c.389_390delAC allele (Figure 1). We further analyzed a collection of 981 DNA samples obtained from phenotypically normal controls and show that the homozygous mutation was absent, with the exception of seven (0.7%) additional individuals (all of whom were from the same Miao village and were later confirmed to be relatives of the two families under study), with the heterozygous deletion in BBS7. Data from all known BBS genes were analyzed; however, sequences were filtered out of our analysis if they did not fit the recessive model of inheritance or if they were not considered a functional mutation. These data are available from the authors upon request.

4. DISCUSSION

In this report, we studied three affected subjects from two Miao families, who were referred to the hospital by their local town health center. Their phenotype assessments are summarized in Table 1. All patients were presented with five established major symptoms of BBS, including obesity, rod-cone dystrophy, renal abnormalities, polydactyly, and learning disabilities. Other

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<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical description of BBS features presented by all three patients</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Sex/Age</strong></td>
</tr>
<tr>
<td><strong>Weight, kg/Height, m</strong></td>
</tr>
<tr>
<td><strong>Pressure, mmHg</strong></td>
</tr>
<tr>
<td><strong>Major BBS phenotypes</strong></td>
</tr>
<tr>
<td><strong>Obesity (BMI, kg/m²)</strong></td>
</tr>
<tr>
<td><strong>Renal anomalies</strong></td>
</tr>
<tr>
<td><strong>Polydactyly</strong></td>
</tr>
<tr>
<td><strong>Learning/comprehension</strong></td>
</tr>
<tr>
<td><strong>Hypogonadism</strong></td>
</tr>
<tr>
<td><strong>Minor features</strong></td>
</tr>
<tr>
<td><strong>Motor skill</strong></td>
</tr>
<tr>
<td><strong>Strabismus</strong></td>
</tr>
<tr>
<td><strong>Dental architecture</strong></td>
</tr>
<tr>
<td><strong>Behavior</strong></td>
</tr>
<tr>
<td><strong>Development delay</strong></td>
</tr>
<tr>
<td><strong>Brachydactyly</strong></td>
</tr>
<tr>
<td><strong>Short neck, low nose bridge</strong></td>
</tr>
<tr>
<td><strong>Diabetes mellitus</strong></td>
</tr>
<tr>
<td><strong>Heart problems</strong></td>
</tr>
<tr>
<td><strong>Hearing loss</strong></td>
</tr>
<tr>
<td><strong>Menstruation in female</strong></td>
</tr>
<tr>
<td><strong>Nystagmus</strong></td>
</tr>
<tr>
<td><strong>Cataract</strong></td>
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<td><strong>Micropenis</strong></td>
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BBS = Bardet-Biedl Syndrome; BMI = body mass index; NA = not available.
minor clinical features were also observed in the three affected individuals. Our patients did not show nystagmus and/or cataracts compared with that in some patients with BBS7 frameshift mutations described in previous reports.15,16

BBS is a ciliopathy involving multiple systems. Eight highly conserved BBS proteins (BBS1, 2, 4, 5, 7, 8, 9, and BBS10) form a complex known as the BBSoome,14 which functions in ciliary membrane biogenesis. BBS7 is an integral part of the BBSoome and physically interacts with the BBS chaperonin complex (BBS6, BBS10, BBS12, and CCT/TRiC family chaperonins).11 Deletion or abnormality of the BBS7 protein can cause structural and functional defects in cilia. Both missense mutations or absent BBS7 can affect the formation of the BBSoome, which can adversely affect various organs in the body.18-20 BBS7 is located on chromosome 4q27 and consists of 19 exons encoding a 715 amino-acid protein. To date, mutations within BBS7 are reported in 4.2% of BBS families,16 Homozygous or compound heterozygous mutations, including nonsense mutations, copy-number variants, and frameshift mutations in BBS7 were identified in affected individuals.22-24 BBS7 was identified as a novel BBS protein in 2003, and since then frameshift mutations including K237fsX60,19 R238EfsX59,20 K237fsX60,26 Q448RfsX13, R238EfsX59,19 and H29QfsX1219 have been reported in the literature. In this study, we identified a homozygous c.389_390delAC (RefSeq NM_176824.2) germline mutation in BBS7 in all BBS patients. This mutation, which resulted in a frameshift, is predicted to lead to premature termination of exon5 (p.Asn130ThrfsTer3), thereby abolishing approximately 81.4% of the wild-type BBS7 protein (133aa versus 713aa) (Ref NP_789794.1). To our knowledge, this mutation has not been previously described in any reported literature. Interestingly, the heterozygous mutation of c.389_390delAC was found not only in unaffected individuel

In conclusion, we have found a mutation c.389_390delAC within BBS7 that is predicted to result in the premature termination of exon5 (p.Asn130ThrfsTer3) and may be essential to the correct formation of BBS7 protein structure. However, there may be a single disease that is “monogenic” in the strict sense of the word. Therefore, further studies are needed to better characterize the genotype-phenotype correlation of the mutation in this report, which is also a limitation of this study.

REFERENCES


Anatomic mapping of the internal spermatic vein via subinguinal varicocelectomy with intraoperative vascular Doppler ultrasound

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Abstract

Background: Varicocele is believed to be a dilated vein of the pampiniform plexus along the spermatic cord. Surgical treatment should be considered in men with a symptomatic varicocele. To date, microsurgical varicocelectomy is the most effective method among various varicocelectomy techniques, according to the current evidence. This study aimed to evaluate the effectiveness of subinguinal varicocelectomy with intraoperative vascular Doppler for symptomatic varicocele and map the distributional trend of spermatic content simultaneously.

Methods: A total of 24 male patients underwent subinguinal varicocelectomy with intraoperative vascular Doppler ultrasound between March 2016 and October 2017, because of symptomatic varicocele or infertility. The numbers, sizes, and location of spermatic vessels in each site were recorded during operation. The visual analogue scale (VAS) score of scrotal pain was also obtained before and after surgery.

Results: The mean number of spermatic veins that were ligated in each spermatic unit was 4.70 (±2.06). The predominant distributional zone of spermatic veins was the medial upper zone on an axial view of the spermatic cord. Fifty-six (44.1%) spermatic veins were found in this zone. Normally, each spermatic cord has 1.33 (±0.61) spermatic arteries. The average VAS score prior to surgery was 1.95 (±0.89) and it decreased to 0.05 (±0.21) after the surgery. Complete resolution of pain was observed in almost all symptomatic patients (95.23%). A significant positive relationship between the number of veins ligated and improvement of VAS score was also noted (p < 0.05).

Conclusion: Subinguinal varicocelectomy with intraoperative vascular Doppler ultrasound is an effective treatment for symptomatic varicocele. The more the internal spermatic veins are ligated, the more the VAS scores are improved. Determining the distributional trend of spermatic content is of great importance in the prevention of iatrogenic injury to the spermatic vessels and vas deferens.

Keywords: Doppler; Spermatic cord; Varicocele; Varicocelectomy

1. INTRODUCTION

Varicocele is an abnormal condition involving enlargement of spermatic veins of the pampiniform plexus along the spermatic cord. The severity of varicocele is defined as grade I, grade II, and grade III on the basis of physical examination. About 20% of adult male population has varicocele, and it is usually asymptomatic. However, 10% of patients with varicocele present with supratesticular pain that may affect daily activities.

Surgical treatment should be considered in men with symptomatic varicocele, oligospermia, and in couples with unexplained infertility. Several methods are available for varicocele repair, such as retroperitoneal high ligation of the internal spermatic vein and subinguinal and laparoscopic varicocelectomy. To date, microsurgical varicocelectomy is the most effective method, with fewer complications and lower recurrence rate according to the current evidence.

Only a few studies have investigated the relationship between the grade and number of internal spermatic veins. However, no studies have mapped the anatomic distribution of internal spermatic veins. Therefore, this study aimed to evaluate the effectiveness of subinguinal varicocelectomy with the assistance of intraoperative vascular Doppler ultrasound for painful varicocele and to determine the relationship between the number of veins ligated and improvement in visual analogue scale (VAS) score. Mapping of the anatomic distribution of the internal spermatic vein within the spermatic cord was also performed in this study.

2. METHODS

2.1. Patient

Between March 2016 and October 2017, a total of 24 male patients who presented with scrotum pain or complained of infertility were diagnosed with symptomatic varicocele or varicocele with infertility. These patients were young adults without medical illness, drug usage, and smoking and drinking habits and came from military service hospital. Most of them performed regular exercise. The grade of varicocele was defined based on physical examination during the first outpatient department visit. Semen analysis was performed if the...
patient has a chief complaint of infertility. Other parameters such as height, weight, and VAS score were also recorded.

Concerning the surgical options, the benefits and disadvantages of retroperitoneal high ligation of internal spermatic veins, laparoscopic varicocelectomy, and subinguinal varicocelectomy with or without microscope assistance were explained to the patients. Before operation, comprehensive physical examination including digital rectal examination and careful palpation of the external genitalia was performed to exclude other concomitant causes such as prostatitis and epididymitis. Patients who underwent subinguinal varicocelectomy without microscope assistance were included in this study.

The study protocol was approved by the Institutional Review Board of Tri-Service General Hospital (TSGHIRB No.:1-107-05-104). Informed consent was waived due to the retrospective nature of the study.

2.2. Technique

Subinguinal varicocelectomy with intraoperative vascular Doppler was performed under spinal anesthesia by well-trained urologists. About 1.5-cm longitudinal incision was made between the external inguinal ring and supra-testicular region. Then, the spermatic cord was identified via fascia incision and blunt dissection. The spermatic cord was adequately separated from the peripheral tissue to decrease its tension and the spermatic cord is carefully manipulated with correct alignment during the operation. Penrose drain was placed below the spermatic cord for support, not to compress it, to keep the target above the incisional wound. With the looseness of the spermatic cord, we could map the location of the vessels precisely. Given the important role of the vas deferens in fertilization and its potential to obscure the detection of intraoperative vascular Doppler, the vas deferens was identified and initially looped with silk to prevent iatrogenic injury and achieve better ultrasound detection on other sites. To further distinguish the nature of uncertain vessels, a vascular Doppler (5 MHz, D.E. Hokanson, Inc.) was used intraoperatively by surgeon to detect the to-and-fro flow of the artery (Figure 1A, B). The vein size was measured using a sterile ruler and was defined as small, medium, and large. Veins with diameters of $<1.0\,\text{mm}$, $1.0\,\text{mm} < 2.0\,\text{mm}$ and $>2.0\,\text{mm}$ were defined as small vein, medium vein, and large vein, respectively. The distribution of vessels was categorized into four quadrant zones on an axial view of the spermatic cord (Figure 2): lateral upper zone (Q1), medial upper zone (Q2), medial lower zone (Q3), and lateral lower zone (Q4). The diameter category, vessel distribution, and location of the vas deferens were recorded.

Patients were discharged on postoperative day 1. Outpatient department follow up was arranged 1 week later. VAS score alleviation was obtained by subtracting the postoperative VAS score from the preoperative VAS score. Improvement of scrotal pain and wound condition were also documented.

2.3. Statistical analysis

The results were expressed as mean ± one standard deviation. All data were recorded as continuous variants, and based on the number of groups, they were analyzed by Student's t-test (one-way ANOVA) with the least significant difference post hoc-testing method to compare the average values. Trend test was performed using one-way ANOVA with linear polynomial regression. Statistical analyses were performed using IBM SPSS Statistics version 18.0 (IBM Corp., Chicago, USA). All statistical tests and $p$ values were two sided, and the level of significance was set to $<0.05\, (**), <0.01\, (***)$, or $<0.001\, (****)$.

3. RESULTS

Between March 2016 and October 2017, 24 patients underwent subinguinal varicocelectomy, and 27 units of spermatic cords were recorded. Of the 24 patients, three patients underwent bilateral subinguinal varicocelectomy. Table 1 shows the demographics of patients with varicocele and distribution mapping of spermatic veins. The average age of patients was $28.07 ± 7.14$ years. The average body mass index was $21.93 ± 2.18$. Eighteen patients underwent subinguinal varicocelectomy because of grade III varicocele with supra-testicular pain. Other indications included infertility in five patients and both pain and infertility in one patient. The average preoperative VAS score was $1.95 ± 0.89$, which decreased to $0.05 ± 0.21$ postoperatively. Almost all symptomatic patients had complete resolution of pain (94.7%). No recurrent varicocele or surgical complication has been noted to date.

Table 1 also shows the distribution, number, and size of the spermatic veins in 27 units of the spermatic cord. The location of the vas deferens and spermatic artery was also recorded. There were 127 internal spermatic veins in these patients. The average count of spermatic vein from each spermatic cord was $4.7 ± 2.06$. The dominant distribution zone of the spermatic veins was Q2; Fifty-six spermatic veins (44.1%) were located in this quadrant. The less distribution zone of spermatic vein was Q3, with only 17 internal spermatic veins (13.4%). In these 27 spermatic units, 25 (92.59%) vasa deferentia were located in Q4. Otherwise, we noticed that vas accompanied arteries often accompanied with the vas deferens in Q4. The average operative time required to complete subinguinal varicocelectomy of each spermatic unit was $72.56 ± 24.76$ min.

Table 2 shows the relationship between VAS score alleviation and clinical parameters of patients with symptomatic varicocele. The VAS score alleviation was divided into three categories: VAS score alleviation of 1, VAS score alleviation of 2, and VAS score alleviation ≥3. Positive correlation with VAS score alleviation was found in vein numbers, vein numbers in Q2, and vein numbers in Q4 ($p < 0.05$).

![Fig. 1](A, Vascular Doppler ultrasound. B, Ultrasound was used to identify left internal spermatic artery under the supine position.)

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Yu et al. J Chin Med Assoc

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116

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4. DISCUSSION

In this study, symptom resolution was reported in almost all patients (94.73%) who presented with supra-testicular pain. Internal spermatic veins were most often located in Q2 (44.1%). A positive correlation was found between VAS score alleviation and the number of intraoperative veins ligated (p < 0.05).

Three patients underwent bilateral subinguinal varicocelectomy, of which one patient had the same number of internal spermatic veins, bilaterally. The other two patients had four internal spermatic veins in the right spermatic cord and five internal spermatic veins in the left spermatic cord. In these three patients, 13 (46.43%) internal spermatic veins were located in Q2, and five vasa deferentia (83.33%) were located in Q4. The findings were similar as mentioned earlier.

There are several approaches for varicocele repair, which includes retroperitoneal, inguinal, and subinguinal. Although microsurgical subinguinal varicocelectomy is the most effective method with fewer complications and lower recurrence rate, this procedure requires microsurgical training. Other therapeutic methods also have been suggested; however, recurrence and hydrocele development are more likely.

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**Table 1**

Demographics of patients with varicocele and distribution mapping of spermatic veins

| Patient number | Age | Weight | BMI | Symptom | Grade | Location | VAS score (Pre/Post/Alleviation) | Quadrant I | Quadrant II | Quadrant III | Quadrant IV | Operation minute | Vein number
|----------------|-----|--------|-----|---------|-------|----------|---------------------------------|------------|------------|-------------|------------|-----------------|-------------
| 1              | 22  | 183/70 | 20.9| Pain    | 3     | Left     | LV,LV LV VAS, A                  | 57         | 3          |             |             |                 |             
| 2              | 36  | 168/61 | 21.6| Pain    | 3     | Left     | LV, LV VAS, A                    | 45         | 2          |             |             |                 |             
| 3              | 17  | 165/57 | 20.9| Pain    | 3     | Left     | LV, LV VAS, A                    | 51         | 2          |             |             |                 |             
| 4              | 27  | 170/65 | 22.5| Pain    | 2     | Right    | 3/0/3 LV SV, SV, SV A            | 65         | 4          |             |             |                 |             
| 5              | 27  | 170/65 | 22.5| Pain    | 3     | Left     | 3/0/3 LV SV, SV, SV VAS, A       | 81         | 5          |             |             |                 |             
| 6              | 34  | 180/80 | 21.6| Pain    | 3     | Left     | 3/0/3 LV LV, LV, SV A            | 73         | 4          |             |             |                 |             
| 7              | 19  | 172/63 | 21.3| Pain    | 3     | Left     | 2/0/2 A, A, LV, SV VAS, A        | 60         | 3          |             |             |                 |             
| 8              | 34  | 180/65 | 20.1| Pain    | 3     | Left     | 1/0/1 LV LV, LV VAS, A           | 39         | 3          |             |             |                 |             
| 9              | 36  | 185/80 | 23.4| Pain    | 3     | Left     | 1/0/1 LV, LV VAS, A             | 54         | 4          |             |             |                 |             
| 10             | 17  | 165/50 | 23.4| Pain    | 3     | Left     | 3/0/3 LV, LV, SV SV VAS, A, LV, SV| 60         | 7          |             |             |                 |             
| 11             | 19  | 176/58 | 18.7| Pain    | 3     | Left     | 1/0/1 SV MV, SV SV VAS, A        | 104        | 4          |             |             |                 |             
| 12             | 17  | 186/72 | 20.8| Pain    | 2     | Left     | 4/1/3 MV, MV, MV VAS, A, LV, LV, MV| 126        | 6          |             |             |                 |             
| 13             | 40  | 179/55 | 17.1| Pain    | 3     | Left     | 1/0/1 LV VAS, A                  | 53         | 3          |             |             |                 |             
| 14             | 22  | 173/64 | 21.4| Pain    | 3     | Left     | 1/0/1 LV, SV VAS, A              | 149        | 5          |             |             |                 |             
| 15             | 26  | 173/66 | 22.1| Pain    | 3     | Right    | 2/0/2 LV, SV, LV VAS, A          | 38         | 5          |             |             |                 |             
| 16             | 22  | 178/82 | 19.6| Pain    | 3     | Left     | 2/0/2 LV, MV, SV VAS, A          | 100        | 4          |             |             |                 |             
| 17             | 23  | 171/74 | 25.3| Pain    | 2     | Left     | 1/0/1 MV, SV, SV A VAS, A        | 80         | 3          |             |             |                 |             
| 18             | 36  | 178/75 | 23.7| Pain    | 3     | Right    | 2/0/2 MV, MV, SV, SV VAS, A      | 68         | 5          |             |             |                 |             
| 19             | 17  | 186/75 | 23.7| Pain    | 3     | Left     | 3/0/3 A LV, LV, SV VAS, A, LV, SV| 82         | 5          |             |             |                 |             
| 20             | 35  | 169/61 | 21.4| Pain    | 2     | Left     | 2/0/2 MV, MV, MV, SV VAS, A      | 65         | 4          |             |             |                 |             
| 21             | 33  | 172/64 | 21.6| Infertility | 2 | Left | 0/0/0 LV, LV, SV, SV SV VAS, A | 63         | 5          |             |             |                 |             
| 22             | 28  | 182/75 | 22.6| Infertility | 3 | Left | 0/0/0 LV, LV, SV, SV, SV VAS, A | 64         | 4          |             |             |                 |             
| 23             | 38  | 175/60 | 19.6| Infertility | 3 | Left | 0/0/0 MV, MV, SV VAS, A          | 93         | 8          |             |             |                 |             
| 24             | 33  | 187/95 | 27.2| Infertility | 3 | Left | 0/0/0 MV, MV, MV MV VAS, A, SV, SV | 75         | 8          |             |             |                 |             

Vein number/percent

| Vein       | 33/26.0% | 56 /44.1% | 17 /13.4 | 21 /16.5% | 1959 | 127 |

A = artery; LV = large vein; MV = medium vein; SV = small vein; VAS = visual analogue scale.
Intraoperative vascular Doppler ultrasonography demonstrated several benefits for subinguinal varicocelectomy. First, Morey and Joel showed that tiny internal spermatic arteries could be localized and identified using intraoperative vascular Doppler ultrasound, which may prevent damage to the small spermatic arteries and decrease the possibility of testis atrophy.7 Second, Marcello et al. demonstrated that intraoperative vascular Doppler allowed more internal spermatic veins to be ligated during operation, and it was considered an excellent tool to improve operative result.8 Third, Liqiang et al. reported that intraoperative vascular Doppler is an effective tool to improve semen quality in patients who underwent operation because of infertility. Compared with subinguinal microscopic varicocelectomy without vascular Doppler, the improvement in sperm motility was more significant in the intraoperative vascular Doppler group owing to the maximal preservation of arterial blood supply to the testis.9

In the present study, we employed subinguinal varicocelectomy without microscope assistance for symptomatic varicocele repair. The mean number of veins ligated was 4.7 ± 2.06. Compared with other studies that applied the microscopic method, the mean number of ligated veins was 6.9 in each spermatic cord.1 This demonstrated that more veins can be ligated using microscopic subinguinal varicocelectomy because of the magnified surgical field that allowed more accurate identification of tiny veins.9

Nineteen patients underwent subinguinal varicocelectomy with intraoperative vascular Doppler ultrasonography because of symptomatic pain. Even without using a microscope, the mean VAS score improved after surgery. Symptom-free was noted in 18 symptomatic patients (94.73%). Furthermore, Min-Che et al. applied the modified subinguinal varicocelectomy in patients with painful varicocele and reported that 90% of their patients had painless status after operation and 10% experienced partial remission of pain.10 In another study, 237 patients underwent microscopic subinguinal varicocelectomy due to scrotal pain, of which 85.6% experienced complete resolution of pain and 6.3% reported partial resolution.11 Comparable therapeutic effect for scrotal pain was seen in both surgical techniques.

No studies have investigated the effect of the number of ligated veins in the improvement of pain score. The present study found a significant positive relationship between the numbers of ligated veins and improvement of VAS scores. To the best of our knowledge, this study is the first to reveal this finding. Haitham et al. demonstrated that the number of ligated veins during operation was not predictive of pain improvement after surgery.12 However, their pain assessment was based on subjective complete resolution of symptoms after operation. In the present study, pain was investigated and assessed using VAS score. In addition, the present study revealed an interesting finding that the degree of VAS score improvement was significantly associated with the number of veins in Q2 and Q4. This meant that the numbers of ligated veins in these two quadrants were more important for symptom relief on the basis of the statistical result. Most engorged veins located in Q2 (44.1%) significantly affect pain relief after surgery. On the contrary, the influence of ligated veins in Q4 on pain relief requires further study. One possibility may be related to the role of veins adjacent to the vas deferens.

No study has investigated the anatomic mapping of spermatic contents. To our knowledge, this study is the first to evaluate the distribution of spermatic veins in Taiwanese patients and map the anatomic distribution of the internal spermatic veins within the spermatic cord. In this study, subinguinal varicocelectomy with intraoperative vascular Doppler was used for patients experiencing painful varicocele or infertility. We found that internal spermatic vein was mainly distributed in Q2 (44.1%). Q2 was the predominant zone of internal spermatic veins and was the most frequent for surgical repair. We also noticed that most internal spermatic arteries and vas deferens-accompanying arteries (86.11%) were often located in the lower quadrants including Q3 and Q4. The finding was compatible with Beck’s study that the testicular artery was adherent to the posterior aspect of large spermatic veins.13 Iatrogenic injury to internal spermatic artery may induce testicular atrophy.2 Therefore, careful attention should be paid to the lower quadrants to prevent unexpected harm to testicular arteries. Moreover, the vas deferens was frequently located in Q4 (92.59%). This distributional property warned us to deal with each quadrant gently.

This study had some limitations. First, this study had a small sample size, which only included 27 spermatic units. Indeed, this was a preliminary study with randomization for limited cases. Although the number of cases was relatively small, this study presents novel and interesting findings, especially in the venous mapping of the spermatic cord. Further studies with more cases are necessary to support these findings. Second, because some infertile patients were lost to follow up, the change in the semen quality after operation was not analyzed.

Subinguinal varicocelectomy with intraoperative vascular Doppler ultrasonography was a reliable method for painful varicocele repair even without microscopic assistance. The more the numbers of internal spermatic veins are ligated, the more the VAS scores are decreased. Determining the distributional trend of spermatic structures helps us repair varicocele more effectively and decrease the probability of iatrogenic injury.

**ACKNOWLEDGMENTS**

We thank Jar-Yi Ho and Chia-Lun Wu for providing assistance in the statistical analysis.
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The effect of high-dose nitroglycerin on the cerebral saturation and renal function in cardiac surgery: A propensity score analysis

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Abstract

Background: The aim of the study was to evaluate the effects of high-dose nitroglycerin administered during cardiopulmonary bypass on the intraoperative cerebral saturation and postoperative serum creatinine concentration in cardiac surgery. Methods: In a retrospective cohort study, a total of 239 patients undergoing cardiac surgery with cardiopulmonary bypass at a tertiary medical center were included. General anesthesia consisted of volatile anesthetic and either intravenous loading of high-dose nitroglycerin (infusion rate 10 to 20 mg·h\textsuperscript{-1} with a total dose of ≥0.5 mg·kg\textsuperscript{-1}) starting from rewarming of cardiopulmonary bypass throughout the end of the surgery (NTG group; N = 96) or without high-dose nitroglycerin (control group; N = 143). Data for intraoperative cerebral saturation and serum creatinine concentrations before and after cardiac surgery were collected. Propensity score method was used to adjust for potential confounders.

Results: Patients receiving high-dose nitroglycerin had significantly lower mean arterial pressure and hematocrit levels during and after cardiopulmonary bypass. The risk of intraoperative cerebral desaturation was left-sided 23.9% versus 38.5% (p = 0.029), right-sided 29.1% versus 35.7% in the NTG and control groups, respectively. The risk of new-onset stroke and postoperative dialysis was 2.1% versus 6.3% and 1.0% versus 3.5% in the NTG and control groups, respectively.

Conclusion: An infusion of high-dose nitroglycerin initiating at rewarming of cardiopulmonary bypass and throughout the post bypass interval may induce hypotension and hemodilution in cardiac surgical patients. Cerebral saturation and renal function were well maintained without increasing the risk of stroke and renal replacement therapy after cardiac surgery with cardiopulmonary bypass.

Keywords: Acute kidney injury; Cardiopulmonary bypass; Cerebral desaturation; Nitroglycerin

1. INTRODUCTION

Cardiopulmonary bypass (CPB) is associated with multiple significant circulatory disturbances, including hypotension, hemodilution, hypothermia, nonpulsatile blood flow, and microemboli. During CPB, endothelial cell dysfunction precipitates a decrease in the production of endogenous nitric oxide (NO), compromising significantly both vascular tone and tissue perfusion.\textsuperscript{1} End-organ hypoperfusion and inherent ischemia are common in the setting of extracorporeal circulation; brain and kidneys are among the most vulnerable organs to the devastating complications.\textsuperscript{2,3}

In addition, although the risk of overt postoperative stroke has decreased from 1.6% to 1.2% in cardiac surgeries since the 1980s,\textsuperscript{4} the impact of overt stroke is profound in terms of worse-adjusted hospital outcomes, longer postoperative hospital stays, and poorer downstream survival.\textsuperscript{5} In addition to microemboli, cerebral hypoperfusion is a major risk factor for brain injury or dysfunction after cardiac surgery, particularly in patients with cerebrovascular disease.\textsuperscript{6} The inflammatory response to surgery and CPB further contribute to cerebral dysfunction.

The incidence of acute renal failure requiring renal replacement therapy (RRT) after uncomplicated cardiac surgery in patients with earlier normal renal function is infrequent (<2%).\textsuperscript{7} However, the incidence of acute kidney injury (AKI) defined by consensus definitions is about 20%–30%.\textsuperscript{8} AKI requiring RRT after cardiac surgery has a profound impact on mortality, and even mild forms of AKI are consistently associated with later development of chronic kidney disease, multiorgan dysfunction, increased mortality, length of stay, and hospital costs.\textsuperscript{9,10}

Administration of intravenous NTG has been proposed to protect against ischemia–reperfusion injuries in a limited number of studies through the mechanism of NO-induced vasodilatation.\textsuperscript{11} Intravenous NTG also reproduces the effect of endogenous late preconditioning.\textsuperscript{12} Nonetheless, effective tissue perfusion can be compromised if blood pressure falls excessively.
under the vasodilatory effect of NTG. Considering the effect of high-dose NTG on the risk of cerebral desaturation and renal injury is relatively underexplored, we conducted this retrospective cohort study applying propensity score analysis to investigate changes in the intraoperative cerebral saturation and postoperative serum creatinine concentration after the treatment of high-dose NTG starting at rewarming phase of CPB in cardiac surgery.

2. METHODS

2.1. Study setting
The study was approved by the medical ethics committee of Taipei Veterans General Hospital, Taipei, Taiwan (TVGHIRB No. 2015-12-018CC). Written informed consent was waived, and all the study materials were anonymized and de-identified before analysis.

At the tertiary medical center, patients undergoing cardiac surgery were frequently given intravenous NTG for the cardio-protective effect. In the past, the regimen of NTG was continuous maintenance with a rate of 0.5 mg·h−1 started instantly after cessation of CPB. A new protocol of NTG treatment was adopted from July 2014 onwards after a review of current literature. There was no significant change in surgical or anesthetic facilities during the study period. The data of the study had been used partly by the authors’ earlier work.11

2.2. Anesthetic management
For each patient undergoing cardiac surgery, serum creatinine concentration was tested 1 day before the surgery. At the operation room, cerebral oximeters (INVOS, Medtronic, MN, USA) were used to measure and record the bilateral cerebral saturation of surgical patients in real time. Baseline cerebral saturation was obtained before anesthetic induction under room air if patients had no cardiopulmonary distress. Patients were given fentanyl 1–2 µg·kg−1 and propofol 1–1.5 mg·kg−1 for induction, and neuromuscular blocking agents to facilitate tracheal intubation with rocuronium 0.8 mg·kg−1 or cisatracurium 0.2 mg·kg−1. During anesthetic maintenance, fentanyl 50–100 µg was given before sternotomy and aortic cannulation. Anesthesia was maintained with sevoflurane 2–3 vol% or desflurane 6–8 vol% in oxygen, with a fraction of inspired oxygen of 0.6–1.0 at the anesthesiologist’s discretion. Arterial blood gas was tested each 5–10 minutes during CPB and 15–30 minutes during other phases of cardiac surgery.

2.3. Protocol of NTG loading
Intravenous administration of NTG was initiated at the rewarming of CPB with an infusion rate of 10 to 20 mg·h−1 and tapered to 5 to 10 mg·h−1 after weaning from CPB. The range of targeted MAP was 40 to 60 mm Hg. If MAP was <40 mm Hg or cerebral oxygen saturation decreased to <80% of the baseline value, the NTG would be tapered accordingly. Typically, NTG was given in a total dose of >0.5 mg·kg−1 during the surgery. Fluids and blood products were first used to maintain systemic blood pressure instead of vasopressors. If cardiac index (CI) was <2.4 l·min−1·m−2, dopamine (3–10 µg·kg−1·min−1) was first given instead of epinephrine or norepinephrine. Milrinone (0.3–1.0 µg·kg−1·min−1) was used at the anesthesiologist’s discretion. After surgery, the infusion rate of NTG was adjusted by the physician of the intensive care unit (ICU) based on patients’ hemodynamics. NTG was typically discontinued within 24 hours after surgery.

2.4. Techniques of cardiopulmonary bypass
HL-30 (Maquet, Rastatt, Germany) roller pumps and Affinity NT (Medtronic, Fridley, MN, USA) oxygenators were used for all patients. Infusion of cardioplegic solutions (15°C to 25°C Custodiol HTK (Koehler Chemi, Alsbach-Haenlein,Germany) or blood (blood to crystalloid ratio 4:1) were performed. The pump flow was an adjusted output of 2.2 l·m−2 of body surface area. The pump flow was decreased to 0.5 l·min−1 in aortic clamping and unclamping. Core temperature was maintained between 32°C and 34°C in valve surgeries and allowed to drift to 34°C in coronary artery bypass grafting (CABG). When the systemic temperature was >36°C, weaning from CPB was attempted.

2.5. Selection criteria of patients
In a review of the anesthetic records of cardiac surgical patients, we included the adult patients undergoing either or both CABG and valve surgery at Taipei Veterans General Hospital between May 2012 and November 2015. Exclusion criteria were emergent surgeries, off-pump surgery, patients with history of preoperative dialysis or congenital heart diseases, and patients with critical missing data. Patients in the NTG group had the anesthetic management and NTG treatment according to the protocol described earlier. One to two controls were sampled for each NTG subject, matched on age (±5 y), sex, type of surgery, and surgeon. Patients in the control group were treated according to the old NTG protocol (an infusion rate of 1–3 mg·h−1) and received a total dosage of NTG <0.5 mg·kg−1 during surgery.

2.6. Outcome measurement
Cerebral saturations were recorded before induction (baseline), after induction, before bypass, 0, 30, 60, 90, 120, 150, and 180 minutes after bypass, before the end of bypass, 30 minutes after the end of bypass, and at the end of surgery. Cerebral desaturation was defined as a relative decrease in regional cerebral oxygen saturation to <80% of the preoperative baseline.

Serum creatinine concentrations were collected at the time point of preoperative baseline, postoperative day (POD) 0 to POD 4 on a daily basis. Acute kidney risk and injury were defined by RIFLE classification,4 namely an increase in serum creatinine 1.5 to 2.0 and 2.0 to 3.0 times baseline, respectively. Creatinine clearance rates before and after the operation were calculated with the Cockcroft-Gault equation.5

Intraoperative hemodynamic parameters were collected, including MAP, heart rate, and body temperature before anesthetic induction, 30 minutes after CPB, and 30 minutes after weaning from CPB. Hematocrit levels were collected from arterial blood gas tests at the time of postinduction (baseline), 15 minutes before cessation of CPB, and 30 minutes after cessation of CPB. Pulmonary artery catheterization was routinely performed with continuous cardiac output monitoring after anesthetic induction in cardiac surgical patients. The values of CI, systemic vascular resistance (SVR), pulmonary vascular resistance (PVR) were collected at the time of baseline, 30 minutes after the end of bypass, and 4 hours after the arrival of ICU.

Postoperative data included the urine output during the first 24 hours of ICU stay, time to extubation, ICU stay, and postoperative hospital stay. In addition, major complications were also recorded, including acute kidney risk, AKI and RRT, new-onset stroke after surgeries, reoperation within 24 hours, readmission due to cardiogenic causes within 3 months, and in-hospital mortality. Postoperative stroke was based on the brain imaging studies, including computed tomography or magnetic resonance imaging and defined as an event within postoperative 2 weeks. The inotropic score was calculated according the following formula: dopamine in µg·kg−1·min−1 + dobutamine in µg·kg−1·min−1 + milrinone in µg·kg−1·min−1 × 10 + epinephrine in µg·kg−1·min−1 × 10. Low-dose dopamine was defined as <3 µg·kg−1·min−1; dobutamine and levsimendan were considered as other inotropic agents. Radiocontrast agents were considered if used within 72 hours before surgery.
2.7. Statistical analysis

Comparisons between the two groups were done with a Pearson’s $\chi^2$ test or Fisher’s exact test for categorical variables and two sample $t$ test or Mann-Whitney $U$ test for continuous variables as appropriate. Propensity score method was used to compensate for the potential difference in baseline attributes between groups and diminish the interaction effect of other variables. The propensity score was obtained by using a logistic regression model, with the addition or omission of high-dose NTG as the dependent variable and all baseline characteristics as independent variables (Appendix 1). The propensity score was then used as the only confounding variable, in association with added or controled high-dose NTG, to estimate the effect of high-dose NTG on the outcomes. A $p < 0.05$ was considered significant. All statistical analyses were conducted with SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). Scientific graphing was performed with Prism version 6.00 (GraphPad Software Inc., San Diego, CA, USA).

3. RESULTS

In the timeframe of the study, 239 patients were available after meeting the selection criteria. In the NTG group, 96 patients (40.2%) received NTG with a total dosage of 0.5 mg·kg$^{-1}$ during cardiac surgery. In the control group, 43 patients (18.0%) were given NTG with a total dosage of <0.5 mg·kg$^{-1}$, and 100 patients (41.8%) had no infusion of NTG during cardiac surgery. There was no significant difference in the patients’ attributes between the two groups (Table 1). The peak NTG infusion rates during rewarming period were median 20 (range 5-40) mg·h$^{-1}$ in the NTG group and 0 (0-10) mg·h$^{-1}$ in the control group. When dividing the patients into three groups, high-dose NTG group (with a total dosage of NTG >0.5 mg·kg$^{-1}$), low-dose NTG group (with a total dosage of NTG >0 and <0.5 mg·kg$^{-1}$) and no NTG group, their baseline characteristics were shown in Appendix 2.

The NTG group had significantly lower MAP during (50 ± 10 versus 57 ± 11 mmHg; $p < 0.001$) compared with control group (Figure 1). Besides, patients in the NTG group had lower hematocrit levels during (25.5 ± 3.4 versus 26.9 ± 3.0%; $p = 0.001$) and after CPB (27.2 ± 3.7 versus 29.2 ± 3.2%; $p < 0.001$) than in the control group (Figure 2). There was no significant difference in the CI and SVR values between groups at the time of postby-pass and ICU stay. However, the PVR after CPB was lower in the NTG group, 110 ± 66 versus and 181 ± 139 dynes·s·cm$^{-5}$ ($p = 0.044$) (Table 2). The effect of high-dose NTG on the hemodynamic change was similar when dividing the patients into three groups (Appendix 3).

The risk of left-sided cerebral desaturation was 23.9% versus 38.5%, $p = 0.023$, and right-sided cerebral desaturation 28.1% versus 35.7% in the NTG and control groups, respectively. NTG subjects had fewer transfusions of fresh frozen plasma (FFP) [0 (0-8) versus 2 (0-12) units FFP; $p < 0.001$] than in the control group. NTG subjects had less perioperative fluid intake (2206 ± 547 versus 2546 ± 841 ml; $p = 0.003$) and less intraoperative inotrope support, including dopamine (32.3% versus 90.9%; $p < 0.001$), epinephrine (6.3% versus 59.4%; $p < 0.001$), norepinephrine (6.3% versus 59.4%; $p < 0.001$), and other inotropes (2.1% versus 18.9%; $p < 0.001$) (Table 3, Appendix 4).

The postoperative peak value of serum creatinine was 1.37 ± 0.65 versus 1.47 ± 0.78 mg·dl$^{-1}$, change of serum creatinine 35.8 ± 38.3 versus 39.5 ± 38.1%, the incidence of acute kidney injury 15.6% versus 19.6% and injury 7.3% versus 8.4%, and RRT 1.0% versus 3.5% in the NTG and control groups, respectively. The postoperative inotropic scores were significantly lower in the NTG group [0 (0-26) versus 4.0 (0-52), respectively; $p < 0.001$]. The risk of major complications was 2.1% versus 6.3% in new-onset stroke, 4.2% versus 4.9% in reoperation, 4.2% versus 10.5% in readmission, and 1.0% versus 7.0% in in-hospital mortality in the NTG and control groups, respectively (Table 4, Appendix 5). Among the cases with stroke, none and five patients had hyperperfusion-type
watershed or lacunar infarction in the NTG and control groups, respectively.

4. DISCUSSION

This study demonstrated that the administration of high-dose NTG during CPB would induce hypotension and hemodilution without elevating the risk of postoperative stroke or renal dysfunction in cardiac surgical patients. The risk of intraoperative cerebral desaturation was lower in the patients treated with high-dose NTG during CPB. Besides, patients treated with high-dose NTG had lower inotropic scores but comparable cardiac performance compared with controls at the arrival of ICU.

Previous studies showed a relative decrease in regional cerebral oxygen saturation of cerebral oximetry to <50% of the preoperative baseline or to absolute levels <50% increase in the risk of adverse postoperative outcomes, including stroke, major organ dysfunction, length of hospital stay, and mortality. In this study, the patients treated with high-dose NTG during CPB had lower risk of intraoperative cerebral desaturation despite their lower MAP and hematocrit levels during and after CPB. The finding is consistent with the prior reports. 15,17

Additionally, the NTG subjects had lower risk of postoperative stroke in the study. The finding is against those reported by Gold and colleagues, who demonstrated that patients with controlled minimal MAP of 50 mmHg were associated with higher risk of neurologic complications in contrast to those with targeted MAP of 80 to 100 mmHg. The sample size of the present study cannot provide enough statistical power to detect a difference in postoperative complications. However, our results suggested that the intravenous infusion of NTG as anesthetic strategy to correct episodes of cerebral oxygen desaturation in high-risk patients seems quite promising and warrants further investigations.

Extracorporeal circulation has detrimental effects on kidneys, including greater reduction in renal perfusion than systemic perfusion, impaired auto-regulation of renal blood flow, hemolysis with release of free hemoglobin, an established nephrotoxicity, and stress hormone and inflammatory responses. 12,13 During CPB, hypotension with nonpulsatile flow promotes renal vasoconstriction and decreases renal blood flow, predisposing kidneys to further perioperative ischemic and nephrotoxic insults. Treatment modality in promoting renal vasodilatation has been tested to prevent development of postoperative renal dysfunction, including dopaminergic agents, prostaglandins, and atrial natriuretic peptide. However, none of the above agents has been proved effective in preventing early AKI.

Pepguero and colleagues demonstrated that an intravenous infusion of NTG before percutaneous coronary intervention was associated with a decreased risk of contrast-induced nephropathy, which shared similar pathophysiologic mechanisms with CPB-induced renal injury. In the current study, patients receiving an infusion of high-dose NTG during CPB had a trend of lower risk of acute kidney risk and injury, RRT and comparable urine output in spite of lower systemic blood pressure and hematocrit levels during and after CPB, although not reaching statistical significance.

Our analysis showed that the patients treated with high-dose NTG had a lower MAP during and after CPB but comparable cardiac output when compared with control group. Some studies have revealed that it is not low pressure during CPB but post-CPB cardiac output that actually best correlates with postoperative renal dysfunction. Furthermore, renal dysfunction is associated with increased renal sympathetic activity. Peterson and colleagues have showed that high-dose infusion of NTG has renal sympathoinhibitory effects in spite of a reduction in both arterial pressure and cardiac

![Fig. 2](image)

**Fig. 2** The hematocrit concentration before, during and after cardiopulmonary bypass in the NTG and control groups. Intraoperative hematocrit concentrations were significantly lower in the NTG group (N = 96) than the control group (N = 143) during and after cardiopulmonary bypass (**p < 0.01; ***p < 0.001).**

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>NTG (N = 96)</th>
<th>Control (N = 143)</th>
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<tr>
<td></td>
<td>Pre-bypass</td>
<td>CPB</td>
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<tr>
<td></td>
<td>Pre-bypass</td>
<td>CPB</td>
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<tr>
<td>MAP, mmHg</td>
<td>95 ± 11</td>
<td>50 ± 10***</td>
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<tr>
<td>HR, bpm</td>
<td>79 ± 15</td>
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<tr>
<td>Temperature, °C</td>
<td>36 (34.3–37.2)</td>
<td>35.6 (32.6–36.9)</td>
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<tr>
<td>Left ScO2</td>
<td>64 ± 13</td>
<td>58 ± 12</td>
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<tr>
<td>Right ScO2</td>
<td>63 ± 14</td>
<td>57 ± 12</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>37.8 ± 4.7</td>
<td>25.5 ± 3.4**</td>
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<tr>
<td></td>
<td>Pre-bypass</td>
<td>Post-bypass</td>
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<tr>
<td></td>
<td>Pre-bypass</td>
<td>Post-bypass</td>
</tr>
<tr>
<td>CI, L·min⁻¹·m⁻²</td>
<td>2.4 ± 0.7</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td>SVR, dyne·s·cm⁻⁵</td>
<td>1175 ± 444</td>
<td>914 ± 481</td>
</tr>
<tr>
<td>PVR, dyne·s·cm⁻⁵</td>
<td>179 ± 126</td>
<td>110 ± 66*</td>
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<td></td>
<td>Baseline</td>
<td>Pre-bypass</td>
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<td></td>
<td>Baseline</td>
<td>Pre-bypass</td>
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<tr>
<td>ACT, s</td>
<td>150 ± 19</td>
<td>543 ± 108</td>
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<tr>
<td></td>
<td>165 ± 31</td>
<td>543 ± 103</td>
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</table>

Values were mean ± SD or median (range).

ACT = activated clotting time; CI = cardiac index; HR = heart rate; MAP = mean arterial pressure; PVR = pulmonary vascular resistance; ScO2 = cerebral saturation; SVR = systemic vascular resistance.

*p < 0.05; **p < 0.01; ***p < 0.001.

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In conclusion, the infusion of high-dose nitroglycerin initiating at rewarming of CPB and throughout the post-bypass interval may induce hypotension and hemodilution. Cerebral saturation and renal function were well maintained without increasing the risk of new-onset stroke or RRT after cardiac surgery with CPB.

ACKNOWLEDGMENTS

Y.H.T. contributed to acquisition of data and manuscript drafting. H.L.W. contributed to data verification. F.W.S. contributed to data collection. K.Y.C. helped review the statistical analysis. C.H.H. and M.Y.T. helped revise the manuscript. C.C.L. contributed to data collection. K.Y.C. helped review the statistical analysis. H.L.W. contributed to data verification. F.W.S. contributed to data collection. Y.H.T. contributed to acquisition of data and manuscript drafting. H.L.W. contributed to data verification. F.W.S. contributed to data collection. K.Y.C. helped review the statistical analysis. C.H.H. and M.Y.T. helped revise the manuscript. C.C.L. contributed to manuscript revision, interpretation of data and gave final approval of the version to be published and agreed to be accountable for the accuracy or integrity of the work.

APPENDIX. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://links.lww.com/JCMA/A12.

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Tai et al. J Clin Med Assoc

124 www.ejcma.org


The usefulness of prophylactic use of acetazolamide in subjects with acute mountain sickness

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Abstract

Background: The mechanisms of acetazolamide (ACZ) in the prophylaxis of acute mountain sickness (AMS) remain unclear. This study evaluated the changes in physiological variables of sleep and heart rate variability (HRV) in subjects with earlier history of AMS who underwent prophylactic treatment of ACZ.

Methods: Nonacclimatized healthy subjects were transported using a bus from 555 m to 3150 m within 3 hours. Polysomnography (PSG) was performed 3 days before ascent (T0), for two consecutive nights at 3150 m (T1 and T2), and 2 days after descent (T3). HRV was measured before sleep and after awakening from T0 to T3. AMS was diagnosed using a self-reported Lake Louise score questionnaire. Subjects found confirmed to have AMS were enrolled in this study. The physiological variables and HRV were compared in AMS subjects without (control group) and with prophylactic ACZ (prophylactic ACZ group).

Results: Thirteen AMS subjects were enrolled. The PSG results were analyzed in eight and HRV were analyzed in nine of the 13 subjects. The prophylactic use of ACZ in the subjects with a history of AMS significantly improved sleep efficiency (p = 0.012) and awakening percentages (p = 0.017) at T1, significantly higher levels of arterial oxygen saturation (SaO2) and lower values of partial pressure end-tidal carbon dioxide tension (PaCO2) at four time points. Furthermore, they had a higher rapid eye movement sleep percentage (p = 0.08) at T2. Prophylactic ACZ treatment significantly increased the normalized unit of high frequency at T1 after awakening (p = 0.028).

Conclusion: Significantly higher quality of sleep, higher SaO2 during sleep, and lower PaCO2 at high altitude were found in the subjects with a history of AMS using prophylactic ACZ before rapid ascent. ACZ may accelerate the acclimatization process for rapid ascents to high altitudes by increasing parasympathetic tone based on HRV analyses.

Keywords: Acetazolamide; Acute mountain sickness; Heart rate variability; Polysomnography

1. INTRODUCTION

Acute mountain sickness (AMS) is the commonest high-altitude illness in nonacclimatized persons arriving at high altitudes. Rapid ascent to altitude above 2500 m may rapidly reduce the arterial oxygen saturation (SaO2) because of the hypobaric hypoxic environment and may induce pathological disorders, such as AMS. The AMS prevalence in Taiwan was 36% at an altitude of 3952 m; 22% at an altitude of 1850–2750 m in Summit County, Colorado, United States, and 42% at an altitude of 3000 m.

Individuals who climb higher than 2500 m may experience worsening symptoms. Additionally, they may experience clouding of consciousness, unsteadiness when walking, and difficulty breathing. If left untreated, cases of high-altitude pulmonary edema or high-altitude cerebral edema are often fatal. The major determinants of AMS are the rate of ascent, the altitude reached, sleeping altitude, and individual genetic susceptibility.

Our earlier study showed that subjects with AMS had lower sleep efficiency, higher awakening percentages, lower central apnea index, longer latency to rapid eye movement (REM), and significantly lower percentages of REM sleep on the first night (T1) at an altitude of 3150 m. Another study by our team showed the effects of rapid ascent on heart rate variability (HRV) of individuals with and without AMS. After rapid ascent, subjects with AMS exhibited no sympathetic excitation, but depressed cardiac parasympathetic modulation. Accordingly, changes in cardiac sympathetic and parasympathetic modulation might play a key role in acclimatization to acute hypobaric hypoxia and/or development of AMS.

Acetazolamide (ACZ), a carbonic anhydrase (CA) inhibitor, is a medication that is commonly used to ameliorate AMS. Metabolic acidosis, which occurs with ACZ, is one of the major stimulant effects on the respiratory system during awake and sleep periods at high altitudes to improve arterial oxygen tension (PaO2) and reduce arterial carbon dioxide tension (PaCO2). Therefore, we investigated the effect of prophylactic use of ACZ on AMS and the changes in physiological variables on sleep and HRV in subjects with a history of AMS subjected to prophylactic treatment with ACZ.
2. METHODS

2.1. Subjects
Adult volunteers with AMS in our earlier study on rapid ascents to high altitudes were eligible for enrolment in this study on the prophylactic use of ACZ. Subjects with severe medical disorders and contraindications to high-altitude exposure and traveling to altitudes above 2000 m in the 2 months before the study were excluded.

We excluded subjects who regularly used hypnotics or sedatives at bedtime and had adequate physical fitness (defined as subjects with sufficient mountaineering experience or who frequently traveled to altitudes exceeding 2000 m).

Subjects who were confirmed to have AMS in our previous study (control group) could participate in this study with prophylactic use of ACZ 250 mg tid 1 day before rapid ascent to high altitudes (prophylactic ACZ group).

2.2. Design
The study protocol (Figure 1) was approved by the Institutional Review Board of Taipei Veterans General Hospital (VGH IRB No. 94-10-14A; 96-01-64A; 97-01-86A), and informed consent was obtained from each subject. Beverages containing caffeine were prohibited for at least 24 hours before measurements. At high altitudes, activity of daily living and mountaineering were allowed during daytime. Subjects complaining of dyspnea were allowed to use supplemental oxygen, but their data were excluded for analysis.

2.3. Polysomnography
Polysomnography (PSG) parameters were measured four times in each subject: three days before ascent (T0), the first two nights following rapid ascent to an altitude of 3150 m (T1 and T2), and 2 days after descent (T3).

PSG was performed using an Alice 4 (Healthdyne, Atlanta, Georgia, USA) by certified sleep technicians. A nasal pressure transducer (PTAF2, Pro-Tech, Woodinville, WA, USA) was used to detect minor changes in airflow and estimate hypopneas.12

PSG recordings were scored according to the standard criteria for the scoring of sleep stages and associated events by certified sleep technicians.13–16 Sleep stages were scored in 30-s sequential epochs and analyzed manually through electroencephalography, electrooculography, and chin electromyography. Apnea was defined as a drop in the peak thermal sensor excursion of ≥90% of the baseline for ≥10 s. Hypopnea was defined as a drop in the nasal pressure signal of ≥30% of the baseline that lasted ≥10 s, resulting in a ≥4% decrease in SaO2 from the pre-event baseline. Apnea–hypopnea index (AHI) was the number of apnea and hypopnea events recorded during the sleep study per hour of total sleep time (TST). Respiratory events could be assessed by measuring chest and abdominal wall movement that those were to demonstrate respiratory effort to distinguish between an obstructive sleep apnea with respiratory effort and central sleep apnea without respiratory effort. A pulse oximeter (for monitoring SaO2, SpO2) was used to record both the pulse and parameters associated with SpO2, including mean and minimum SpO2 and desaturation index (≥4% decrease in SpO2, recorded during the study per hour of TST).

2.4. Partial pressure of end-tidal carbon dioxide
Partial pressure of end-tidal carbon dioxide (PETO2; mmHg) was measured on the day of ascent (i.e., at sea level) and following rapid ascent to an altitude of 3150 m (T1 and T2), and 2 days after descent (T3).

Fig. 1 Flowchart of study subjects and study protocol.
performed before sleep (BS) at night and after awakening (AA) on the next morning at high altitudes (T1 and T2) by using a tidal wave handheld capnography (Model 610; Novametrix Medical Systems, Inc., Wallingford, CT, USA). The subjects were tested in a seated position and were instructed to breathe normally for 60 s through an airway adapter with a mouthpiece. The median value was recorded once the breathing pattern reached a steady state. Data on SpO₂ and pulse rate (PR) at awakening were obtained simultaneously.

2.5. Heart rate variability
Short-term HRV was measured at BS and AA using a handheld instrument (Chenshinh, DailyCare BioMedical Inc., Zhongli, Taiwan). After resting in a supine position for 10 min, 15-min electrocardiogram (ECG) was recorded in the same position at BS. Subjects were asked to relax (but not to fall asleep) and breathe naturally. On the subsequent morning, the subjects again underwent 15-min ECG at AA but before they got out of bed.

Autonomic cardiovascular function was evaluated through rate-interval analysis (RRI) analysis using built-in software. A 5-min ECG waveform was conducted and artifacts, large transients, or signal fluctuations were not included in the calculation of HRV was obtained to measure consecutive RRI. Atrial and ventricular arrhythmias and sinus pauses were excluded; the consequent missing data were replaced with interpolated beats derived from the nearest valid data. If more than 5% of the beats were deleted, the data were excluded.

Among the time and frequency domains, were analyzed in accordance with the HRV guidelines of the Task Force.¹⁷ Time-domain measurements included the mean RRI, standard deviation of all normal to normal (NN) intervals, the square root of the mean squares of differences of successive NN intervals (RMSSD), the number of interval difference of successive NN intervals more than 50 ms (NN50), the proportion derived from the total number of NN intervals (pNN50).

Fast Fourier transform spectrum was used to analyze frequency domains as total power (TP): 0.01–0.4 Hz, very low frequency (VLF): 0.01–0.04 Hz, low frequency (LF): 0.04–0.15 Hz, and high frequency (HF): 0.15–0.4 Hz. The normalized unit of LF (LFnu) and the normalized unit of high frequency (HFnu) were calculated, as LF/TP – VLF x 100 and HF/TP-VLF x 100, respectively.

2.6. Evaluation of AMS
Self-reported Lake Louise scores (LLSs) were used to diagnose AMS. Subjects were asked to complete the LLS questionnaire before sleeping and AA, and the highest score during the stay at high altitudes was considered the final score. Subjects were diagnosed with AMS if their LLSs were ≥3 in the presence of a headache with one or more of the following gastrointestinal symptoms (nausea, anorexia, or vomiting), dizziness, difficulty sleeping, and fatigue or weakness.¹⁸,¹⁹

2.7. Statistical data analysis
Data reported were mean ± standard error of the mean (SEM) in the HRV variables, and mean ± standard deviation (SD) in the other variables. Nonparametric tests were used to analyze the variables because the dispersion of the data was wide. Differences between the subjects with AMS who did not use ACZ for prophylaxis and the subjects who did use ACZ for prophylaxis were assessed using the Wilcoxon signed-rank test. For intragroup comparisons, the differences in HRV and P_{CO2} between BS and AA were evaluated using the Wilcoxon signed-rank test. The differences in PSG and HRV parameters over the four time points were examined through pairwise comparisons by using the Wilcoxon signed-rank test and Bonferroni correction. Statistical analyses were conducted using the PASW (Predictive Analytics Suite Workstation) 18.0 statistical software package (SPSS Inc., Chicago, USA). Significance was set at p < 0.05 (2-tailed) except for variables applicable to the Bonferroni correction. For intragroup comparisons, significance was set at p < 0.0083 (0.05 ÷ 6).

3. RESULTS

3.1. Subject characteristics
Thirteen AMS subjects were included in the analysis (three men and 10 women; age range: 23–60 years [mean ± SD, 45.2 ± 12.4 years]). AMS was persistent in 1 (female) of the 13 (7.7%) subjects after using ACZ for prophylaxis. However, the severity was reduced remarkably. The time between the subjects participated the study diagnosed to have AMS and the study on the prophylactic use of ACZ were separated in time by 4–53 months (mean ± SD, 17.2 ± 14.0 months).

3.2. The effect of prophylactic ACZ on PSG parameters in subjects with AMS
Three subjects with AHIs ≥5 at T0 and two subjects who experienced equipment failure were excluded. Ultimately, eight subjects were included in the PSG analysis.

The serial changes in sleep architecture in eight subjects with AMS indicated that the subjects with prophylactic use of ACZ exhibited higher-quality sleep. Compared with the data without ACZ, the data with ACZ exhibited higher sleep efficiency (73.0 ± 14.5 vs 85.8 ± 8.2, p = 0.012) and lower awakening percentages (24.1 ± 14.6 vs 11.1 ± 6.6, p = 0.017) at T1. Moreover, greater REM sleep percentages (11.3 ± 5.9 vs 15.5 ± 6.8, p = 0.05) at T2 was found in the subjects with prophylactic ACZ. Shortening of the latency to REM sleep measured at T1 was found in the subjects with prophylactic use of ACZ.

Over time, changes in respiratory events and oxygenation-related parameters were observed in eight subjects with AMS. The use of prophylactic ACZ improved SaO₂. In addition, significantly higher mean SpO₂ (80.5 ± 6.4 vs 86.9 ± 2.6, p = 0.025) and significantly higher minimum SpO₂ (71.5 ± 8.7 vs 78.5 ± 3.3, p = 0.027) at T1 were found in the subjects with prophylactic ACZ. At a high altitude (T1 and T2), the values for AHI, hypopnea index, and desaturation index were slightly higher than those at sea level (T0 and T3).

3.3. The effect of prophylactic ACZ on P_{CO2} in subjects with AMS
All 13 subjects were included in the P_{CO2} analysis. P_{CO2} was measured at five time points: sea level, T1 BS, T1 AA, T2 BS, and T2 AA. The P_{CO2}, SpO₂, and PR values were comparable despite the prophylactic use of ACZ (Figure 2). Stepwise decreases were observed in the level of P_{CO2} from sea level to T2 AA. Prophylactic use of ACZ could significantly decrease the levels of P_{CO2} for all recorded times, and significantly increase the levels of SpO₂ at four time points, except for T1 AA. Moreover, prophylactic use of ACZ might lower the levels of PR slightly.

3.4. The effect of prophylactic ACZ on HRV parameters in subjects with AMS
Two subjects with more than 5% missing beats and two subjects recorded with ventricular premature contraction were excluded. Ultimately, nine subjects were included in the HRV analysis. Almost all parameters indicative of parasympathetic tone, including RMSSD, NNN50, pNN50, and HF, increased at T1 AA rather than T1 BS in subjects with prophylactic use of ACZ (Figure 3). In addition, the subjects with prophylactic use of ACZ had significantly higher HFnu at T1 AA (p = 0.028), RMSSD (p = 0.015), and pNN50 (p = 0.021), and the LF/HF ratio (p = 0.038) significantly decreased at T3 AA.

At a high altitude, the AA HFnu of parasympathetic tone significantly decreased compared with that BS; however, the LF/HF ratio for sympathovagal balance increased significantly. Throughout T0–T3 AA, the subjects with prophylactic use of
ACZ had consistently higher levels of all time-domain parameters and all spectral segments (namely TP, VLF, LF, and HF) than at T0–T3 BS. The AA HFnu were lower than the BS HFnu, with significant differences observed at T1 (46.0 ± 7.6 vs 29.9 ± 5.8, \( p = 0.021 \)) and T2 (40.8 ± 6.6 vs 18.8 ± 4.0, \( p = 0.008 \)); moreover, the levels of LFnu were lower at T2 AA than at T2 BS. At sea level (T0), the AA LF/HF ratios were slightly lower than the BS LF/HF ratios. However, at T1 (1.1 ± 0.3 vs 2.1 ± 0.6, \( p = 0.038 \)) and T2 (1.4 ± 0.6 vs 2.9 ± 0.9, \( p = 0.036 \)), the AA LF/HF ratios were significantly higher than the BS LF/HF ratios.

BS, the data of LFnu of sympathetic tone and LF/HF ratio nonsignificantly decreased at T1 more than those did at T0 but increased at T2 more than those did at T1. By contrast, the data of HFnu of parasympathetic tone slightly increased at T1 more than those did at T0 but decreased at T2 more than those did at T1. Stepwise decreasing trends were noted for all time-domain parameters and all spectral segments from T0 to T2 BS. At a high altitude, the data of LFnu increased at a high altitude, and the values remained slightly higher at T1 AA than at T2 AA. The LF/HF ratio gradually increased from T0 to T2 AA. All HRV values were similar between T0 and T3 AA.

3.5. Side effects of ACZ in subjects with AMS

We estimated the side effects from ACZ in 13 subjects with prophylactic use of ACZ. The side effects of ACZ were noted including numbness, gastrointestinal discomfort, dizziness, leg edema, and headache. However, the side-effects were uncommon, mild, and well tolerated by the subjects.

4. DISCUSSION

To the best of our knowledge, this is the first pilot study to evaluate the effect of rapid ascent to a high altitude for two consecutive days on sleep and HRV changes and to compare the clinical relevance of prophylactic use of ACZ in particular in those with a history of AMS using the same protocol of rapid ascent to a high altitude. We assessed sleep quality through full-night PSG, so that we could understand more about the sleep architecture and respiratory events. We demonstrated that subjects with a history of AMS treated with prophylactic ACZ could improve sleep quality. They had higher sleep efficiency, lower awakening percentages, higher mean \( \text{SpO}_2 \), and higher minimum \( \text{SpO}_2 \) at T1.

![Fig. 2](image_url)
At T2, they had higher percentages of REM sleep. Furthermore, the variables indicative of parasympathetic activity based on an analysis of HRV (NN50, pNN50, and HF) increased at T1 AA compared with those at T1 BS. By contrast, sympathetic activity showed no significant change between T1 AA and T1 BS. ACZ was confirmed to reduce symptoms of AMS and facilitate acclimatization. However, the actual mechanisms remain unclear and require further investigations to elucidate. ACZ increases the excretion of bicarbonate in the proximal tubule of the kidney, resulting in metabolic acidosis and the balance of hyperventilation-induced respiratory alkalosis. ACZ stimulates ventilation, improves oxygenation, and accelerates the body’s acclimatization process. A study reported that the differences in mean AMS scores over time exhibited a statistically significant decline in the ACZ group versus the placebo group, and ACZ effectively reduced the symptoms of AMS over a 24-h period after arrival at 3630 m. ACZ reduced the incidence and severity of sleep-disorder breathing and was associated with improvements in SaO₂, resulting in the enhancement of sleep quality. In this study, the subjects with an AHI of ≥5 were excluded. Notably, ACZ still benefited sleep quality in subjects with a history of AMS without sleep-disorder breathing. Our results indicated that prophylactic use of ACZ could significantly improve mean and minimum SpO₂, in subjects with a history of AMS on the first night at a high altitude of 3150 m. Furthermore, the subjects with a history of AMS who were subjected to prophylactic use of ACZ had significantly higher sleep efficiency, lower awakening percentages, and more REM sleep. Our previous study indicated that REM sleep delay and reduction was observed in subjects who were not acclimatized to acute hypobaric hypoxia. ACZ has been proven to reduce periodic breathing that is common in high-altitude sleep disturbance and increase sleep quality.

Teppema et al. enrolled nine healthy volunteers to measure the effect of ACZ (250 mg orally every 8 h for 3 days) on the dynamic ventilator response to stepwise changes of PETCO₂. They showed that compared with placebo group, resting ventilation significantly increased from 12.22 ± 2.41 to 14.01 ± 1.85 L/min, resulting in a significant decrease in PETCO₂ from 40.0 ± 4.7 to 33.3 ± 3.5 mmHg in the ACZ group. Ventilation increased after

Fig. 3 The square root of the mean of the sum of the squares of differences between adjacent normal to normal (NN) intervals (RMSSD, A), the NN intervals differing by more than 50 ms (NN50, B), the NN50 count divided by total number of NN intervals (pNN50, C), high frequency (HF, D), the normalized unit of HF (HFnu, E), the normalized unit of LF (LFnu, F), and LF/HF (G) measured at different time points in the subjects with acute mountain sickness (AMS) with and without prophylactic use of acetazolamide (ACZ). † indicated comparisons within groups: *p < 0.0083 vs T0; †p < 0.0083 vs T1; ‡p < 0.0083 vs T2.
the administration of ACZ, resulting in a decrease in $P_aCO_2$ and an increase in $P_aO_2$. In agreement with the previous study, our results indicated that $P_aCO_2$ progressively decreased when the subjects rapidly ascended to a high altitude. The data of $P_aCO_2$ were significantly lower in subjects with a history of AMS with prophylactic use of ACZ than in the same subjects without the use of prophylactic ACZ (Figure 2).

In our previous report, we determined that parasympathetic activity (RMSSD, NN50, pNN50, and HFnu) decreased in subjects with a history of AMS but significantly increased in those without AMS on the first morning after a rapid ascent to a high altitude. Furthermore, in the present study, the prophylactic use of ACZ made the most marked distinction between the AMS subjects without (control group) and with (prophylactic ACZ group) prophylactic use of ACZ was the conflicting trends in the changes in parasympathetic tone after the first overnight sleep (Figure 3). In the current study, almost all parameters of parasympathetic activity increased at T1 AA compared with those at T1 BS in subjects with AMS who underwent prophylactic use of ACZ. The prophylactic use of ACZ in subjects with previous AMS led to increased parasympathetic activity. Some studies indicated that acclimatization of high altitude could be characterized by recovery of parasympathetic tone. We found that prophylactic use of ACZ accelerated the high-altitude acclimatization process by increasing parasympathetic tone.

The limitations of this study were as follows. First, this study featured an unevenly distributed range of ages. Wang et al. reported an AMS prevalence of 36% in trekkers at Jade Mountain, Taiwan; Bloch et al. reported an AMS incidence of 37.5% in children ascending rapidly to 3450 m: the incidence of AMS in these children was almost identical to that of adults. Second, a sex imbalance was observed, with a female dominance. However, susceptibility to AMS does not differ between men and women. Third, each AMS subjects without (control group) and with (prophylactic ACZ group) ACZ use was studied in a wide range of period 4–53 months (mean ± SD, 17.2 ± 14.0 months). Fourth, the sample size for subjects with below-average fitness was relatively small. Fifth, the severity of AMS might have been underestimated because five subjects with severe AMS who used supplemental oxygen were not included. However, the results
of this study provide valuable information about the effect of ACZ on the changes in sleep and HRV after rapid ascent to a high altitude and during the two consecutive days at the same altitude in the subjects with a history of AMS. Moreover, this study identified the differences between AMS subjects without (control group) and with (prophylactic ACZ group) prophylactic use of ACZ.

In conclusion, significantly higher quality of sleep, higher SpO2 during sleep, and lower P ETCO2 at a high altitude were found in the subjects with a history of AMS using prophylactic use of ACZ before rapid ascent. ACZ may accelerate the acclimatization process for rapid ascents to high altitudes by increasing parasympathetic activity based on HRV analyses.

ACKNOWLEDGMENTS

This work was supported by grants from Taipei Veterans General Hospital (TVGH96-C1-106 and TVGH98-C1-136) and National Science Council (NSC99-2314-B-010-033-MY2).

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Prognostic factors related to intratumoral hemorrhage in pediatric intracranial germ cell tumors

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Abstract

Background: Certain types of pediatric intracranial germ cell tumors (PIGCTs) are prone to intratumoral hemorrhaging (TH) and associated with poor survival outcome. However, the impact of TH on the functional prognosis of patients with PIGCTs has not been well studied. This study aimed to evaluate the clinical and MR findings in PIGCT patients with TH to identify the factors related to patient survival and functional outcome.

Methods: This study included 17 patients diagnosed with PIGCT and TH between 2002 and 2016 and evaluated TH-associated clinical and MR findings. The modified Rankin scale (mRS) was used to evaluate functional outcome, which was poor when mRS ≥ 3. The volumes of hematomas and tumors were manually tracked within each brain magnetic resonance imaging slice.

Results: Among the 17 patients, 6 (35.3%) died and 9 (52.9%) had poor functional outcome. Regarding the functional outcome, the mean hematoma volume to tumor volume ratio (HTVR) was 8.5 ± 3.9% in the favorable outcome group and 42.3 ± 27.8% in the poor outcome group (p = 0.001). For the survival outcome, the mean HTVR was 15.7 ± 16.1% in the living group and 46.0 ± 31.5% in the deceased group (p = 0.016). The cutoff point of the receiver operating characteristics curve for HTVR to predict death and poor functional outcome was 19.27% and 16.8%, respectively.

Conclusion: Our study demonstrated that patients with larger HTVR had significantly worse functional and survival outcomes than those with smaller HTVR. We suggest that early and aggressive treatment for PIGCTs in patients with large HTVR can improve their long-term prognosis.

Keywords: Brain; Germ cell tumors; Pediatric MRI; Tumoral hemorrhage

1. INTRODUCTION

The occurrence of pediatric intracranial germ cell tumors (PIGCTs) is significantly higher in Asia than in the West.1,2 Survival of patients with PIGCTs is mainly determined by their histology types.3 The 10-year survival rate of patients with pure germinoma can be as high as 92.7%. In contrast, patients with certain types of germ cell tumors (yolk sac tumor, embryonal carcinoma, or choriocarcinoma) have 3-year survival rates as low as 27.3%.4

Intracranial tumoral bleeding is rare in pediatric patients. Patients with choriocarcinoma and nongerminomatous germ cell tumors (NGGCTs) with choriocarcinoma elements are notorious for being at high risk of tumoral hemorrhage (TH) and poor survival.1 However, the influence of TH on functional and survival outcomes of PIGCTs has not been well studied.2 The purpose of this study was to identify the factors affecting functional and survival outcomes of PIGCT patients with TH. To achieve this goal, we evaluated clinical and magnetic resonance imaging (MRI) findings in these patients. By more aggressively managing these factors, we would expect to improve the long-term prognosis of these pediatric patients.

2. METHODS

The protocol of this retrospective study was approved by our institutional review board, giving us access to the clinical records and radiological images of the patients with PIGCTs.

2.1. Patients and clinical findings

There were 116 pediatric patients diagnosed with intracranial germ cell tumors in our institute between 2002 and 2016. Seventeen of these patients also had TH at the time of their initial diagnosis. TH was diagnosed by pathological examination in 13 patients and by clinical and MR results in 4 patients. Clinical findings, including demographic features, chief clinical symptoms, final diagnosis, and functional and survival outcomes of treatment, were evaluated (Table 1). Any recurrence of diseases or complications of treatment were recorded. The
Demographic features of the 17 pediatric patients with hemorrhagic intracranial germ cell tumors

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number</th>
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<tr>
<td>Age, y</td>
<td>11.1 ± 4.3 (4-19)</td>
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<tr>
<td>Sex</td>
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<tr>
<td>Initial symptoms</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Blurred vision</td>
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<td></td>
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<td></td>
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<td></td>
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<td>Tumor MRI features</td>
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<td>T1WI</td>
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<td></td>
<td>T2WI</td>
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<tr>
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<td>Early subacute</td>
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<tr>
<td>Tumor volume, cm³</td>
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<tr>
<td>Hematoma volume, cm³</td>
<td>6.5 ± 7.7 (0.1-25.5)</td>
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<tr>
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<tr>
<td></td>
<td>Dead</td>
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<tr>
<td>Survival time, y</td>
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<td></td>
<td>mRS ≥ 3</td>
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</table>

aAcute symptoms indicate clinical symptoms that are due to increased intracranial cerebral pressure or intracranial herniation. Stage of hematoma: hyperacute, 1 (T1 iso, T2 high); acute, 2 (T1 iso, T2 low); early subacute, 3 (T1 high, T2 low); late subacute, 4 (T1 high, T2 iso); chronic, 5 (T1 and T2 low).

2.2. MRI findings

The MR images of the brain and spine were acquired on 1.5T MR scanners (Signa HD & Excite TwinSpeed; GE Healthcare, Waukesha, WI). The sequences included a T1-weighted image, FLAIR, T2-weighted image, and 3D-contrast-enhanced T1-weighted image. Diffusion MR images with b = 0 and b = 1000 s/mm² were available for 14 patients. All patients also underwent contrast-enhanced spinal MRI to elucidate whether any seeding by the tumor had occurred during the initial diagnosis and subsequent follow-up period. The intervals between acute symptoms onset and the time of the MR image examination were recorded. The pretreatment scans were analyzed. The clinical and MR follow-ups were conducted every 3 months in the first 2 years after treatment and every 6 to 12 months thereafter.

Imaging studies were retrospectively reviewed by two neuroradiologists (with 3 and 20 years experience), and final decisions on diagnosis, tumor location, volume, component, signal change, enhancement characteristics, and the location and stage of the hematoma were reached by consensus. The tumor was defined as “solid” when <25% of its volume was cystic; “cystic” when >75% of its volume was cystic; and “mixed” when between 25% and 75% of its volume was cystic. Intratumoral hemorrhaging was diagnosed from clinical and MR findings and/or pathological examination. On the basis of MR gradient-echo imaging or susceptibility-weighted imaging, intra-THs were classified as either intracystic or solid. Intracystic hemorrhage was indicated by blood inside cystic structures, including fluid-fluid levels within smooth-in-contour enhanced cyst walls (Figure 1). Solid hemorrhage was indicated by hematoma within the solid portion of the tumor (Figure 2). Hemorrhage was classified according to temporal change on T1/T2-weighted images into five stages: hyperacute (T1 iso, T2 iso to high), acute (T1 iso, T2 low), early subacute (T1 high, T2 low), late subacute (T1 high, T2 high), and chronic (T1 and T2 low). If the MR signals of hemorrhage stage were mixed, the correlation of the most recent stage with clinical symptoms would be analyzed. The volume of hematomas and tumors was manually traced in each brain MRI slice and was calculated by multiplying the thickness of the slice by the areas of the hematoma and tumor, respectively. If there were multiple sites of hemorrhage, the total volume of the hematomas would be calculated by summing the volumes of hemorrhage at these sites. The total hematoma volume was also expressed relative to total tumor volume for better comparison between different tumor sizes and different ages of the children. We used the hematoma volume to tumor volume ratio (HTVR) for evaluating the functional and survival outcomes.

2.3. Statistical analysis

The statistical analysis was performed using SPSS for Windows (version 18). For univariate analysis, the nonparametric methods and χ² test were used to assess differences in categorical variables. Receiver operating characteristic (ROC) curves were used to explore the characteristics of diagnostic tests by graphing the false positive rate (1-specificity) on the horizontal axis and the true positive rate (sensitivity) on the vertical axis with various cutoff values. A p value of 0.05 was considered to indicate a statistically significant difference among the test populations.

3. RESULTS

3.1. Patients and clinical findings

The demographic features of the 17 PIGCT patients with TH are shown in Table 1. Their mean age at diagnosis was 11.1 years (11.1 ± 4.3 [4-19]). Their diagnoses included germinoma (n = 7 [41.2%]), mixed GCT (n = 8 [47%]), yolk sac tumor (n = 1 [5.9%]), and choriocarcinoma (n = 1 [5.9%]).

modified Rankin scale (mRS) was used to assess functional outcome. “Poor outcome” was defined as mRS ≥ 3, and “favorable outcome” was defined as mRS ≤ 2.6

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Acute symptoms, such as signs of acutely increased intracranial pressure, were observed in 10 (58.8%) patients, and non-emergent symptoms, such as diabetes insipidus or lethargy, were observed in 7 patients. The mean follow-up period was 5.6 ± 4.2 (0.8-15.2) years. The overall survival rate and 5-year survival rate for the 17 patients were 11/17 (64.7%) to date and 12/17 (70.6%), respectively. Within a mean period of 3.3 ± 2.9 (0.8-8.8) years, four patients died from tumor recurrence, one from tumor progression, and one from a radiation-related secondary tumor. The functional outcomes of all 17 patients were characterized as either poor in 9 (52.9%) or favorable in 8 (47.1%).

3.2. MR findings

The MR findings of the 17 PIGCT patients with TH are presented in Table 1. The average interval between symptom onset and MR examination was 5.9 days (5.9 ± 4.0 [2-14]). Subarachnoid seeding or metastasis was noted initially in six cases (35.3%). The mean volumes of the tumors and hematomas were 23 cm$^3$ (23.0 ± 15.7 [1.8-53.7]) and 6.5 cm$^3$ (6.5 ± 7.7 [0.1-25.5]), respectively. The mean HTVR was 26.4% (26.4 ± 26.4 [4.8-88.6]).

Hematomas were intracystic for nine patients (52.9%) and within solid tumor for eight (47.1%). In one case, the TH extended to the solid tumor surface and entered the ventricle. Two of the 17 (11.8%) patients had multiple stages of hematoma, which indicated repeated bleeding. The mean tumor volume, mean hematoma volume, and HTVR were 18.7 cm$^3$, 7.9 cm$^3$, and 29.1% for the germinoma group and were slightly but not statistically significantly larger in the NGGCT group ($p = 0.380, 0.360, and 0.631, respectively$).

3.3. Statistical analysis

3.3.1. Functional and survival outcomes

The statistical analysis of factors potentially affecting functional and survival outcomes is shown in Table 2.

Regarding clinical functional outcome, the poor outcome group had significantly larger mean hematoma volume (10.4 ± 8.9 versus 2.2±1.8 cm$^3$ in the favorable group; $p = 0.021$) and mean HTVR (42.3 ± 27.8% versus 8.5 ± 3.9%; $p = 0.001$). Regarding the survival outcome, the deceased group had significantly larger mean HTVR (46.0 ± 31.5% versus 15.7 ± 16.1% in the living group; $p = 0.016$).

3.3.2. ROC curve analysis

The results of ROC curve analysis for predicting poor outcome are shown in Table 3. For hematoma volume, the estimated area under the curve was 0.83 ($p = 0.021$) for predicting the poor functional outcome (mRS $\geq 3$), and the cutoff value was estimated to be 5.6 cm$^3$, with 67% sensitivity and 100% specificity. The areas under the curve for HTVR as an indicator of poor functional and survival outcome were 0.97 ($p = 0.001$) and 0.86 ($p = 0.012$); the cutoff value, sensitivity, and specificity were 16.8%, 89%, and 100% for the patients with mRS $\geq 3$ and 19.3%, 83%, and 82% for the deceased group, respectively.

4. DISCUSSION

The incidence of spontaneous bleeding of PIGCT has been rarely reported, and it is in the range of 9.4% to 14.3%. In the study by Liang et al, hemorrhage was identified in the tumors of 13 NGGCT patients, whereas none was observed in
the tumors of 19 germinoma patients. We found 17 cases of PIGCT with TH (7 with germinomas and 10 with NGGCTs) in a total of 116 cases of PIGCT (14.7%). Although NGGCTs (compared with germinomas) had larger tumor and hematoma volumes, and a larger percentage of them were hemorrhagic, the difference in size was not statistically significant. This result suggested that spontaneous bleeding can occur in both germinomas and NGGCTs.

Patients with germinomas have better prognosis than those with NGGCTs.4,11 The 5-year survival rate of patients with pure germinomas ranges from 89.2% to 93.3%.11,12 NGGCTs are less radiosensitive than germinomas, and the 5-year survival rates are lower, that is, 50% to 70%.11,12 In our studies, the 5-year survival rate was 85.7% for germinoma patients with TH and 60% for NGGCT patients with TH. The survival outcomes of both groups were similar to that reported in the literature. These findings suggested that TH by itself does not significantly influence survival. Consequently, we looked at other potential outcome predictors, such as hematoma volume.

It was unclear in the past whether TH influences the functional and survival outcomes of patients with PIGCT. In this study, functional and survival outcomes were significantly worse for patients with larger HTVR. Patients with larger hematoma volume also had worse functional outcomes. Poor functional outcome was predicted when hematoma size was >5.6 cm³, or mean HTVR was >16.8% (Table 3). These findings suggested that the hematoma volume had a significant influence on the outcomes of PIGCT patients with TH. Therefore, we recommend using hematoma volume and HTVR for predicting functional and survival outcomes in these patients. These results might be explained by acute hematoma expansion causing irreversible direct damage to the adjacent brain tissue, or tumor-induced compression of the brain causing acute increased

### Table 2

<table>
<thead>
<tr>
<th>Survival outcome</th>
<th>Clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alive (N = 11)</strong></td>
<td><strong>Death (N = 6)</strong></td>
</tr>
<tr>
<td>Tumor pathology</td>
<td></td>
</tr>
<tr>
<td>Germinoma</td>
<td>6</td>
</tr>
<tr>
<td>Nongerminoma</td>
<td>5</td>
</tr>
<tr>
<td>Tumor location</td>
<td></td>
</tr>
<tr>
<td>Midline (pineal and suprasellar regions)</td>
<td>7</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
</tr>
<tr>
<td>Tumor component</td>
<td></td>
</tr>
<tr>
<td>Solid</td>
<td>4</td>
</tr>
<tr>
<td>Cystic and mixed</td>
<td>7</td>
</tr>
<tr>
<td>Initial seeding</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>4</td>
</tr>
<tr>
<td>N</td>
<td>7</td>
</tr>
<tr>
<td>Acute symptoms</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>6</td>
</tr>
<tr>
<td>N</td>
<td>5</td>
</tr>
<tr>
<td>Stage of hematoma</td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>3</td>
</tr>
<tr>
<td>Subacute</td>
<td>8</td>
</tr>
<tr>
<td>Location of intratumoral hematoma</td>
<td></td>
</tr>
<tr>
<td>Intracystic</td>
<td>7</td>
</tr>
<tr>
<td>Solid</td>
<td>4</td>
</tr>
<tr>
<td>Tumor volume, cm³</td>
<td>23.7 ± 17.4</td>
</tr>
<tr>
<td>Volume of hematomas, cm³</td>
<td>4.5 ± 7.2</td>
</tr>
<tr>
<td>Hematoma/Tumor ratio, %</td>
<td>15.7 ± 16.1</td>
</tr>
</tbody>
</table>

**p < 0.05, **p < 0.01.

mRS = modified Rankin scale; p = p value for χ², Fisher exact test, or t-test.

### Table 3

<table>
<thead>
<tr>
<th>Cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor clinical outcome (mRS = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor volume, cm³</td>
<td>23.2</td>
<td>0.44</td>
<td>0.75</td>
</tr>
<tr>
<td>Hematoma volume, cm³</td>
<td>5.6</td>
<td>0.67</td>
<td>1</td>
</tr>
<tr>
<td>Ratio of hematoma/tumor volume, %</td>
<td>16.8</td>
<td>0.89</td>
<td>1</td>
</tr>
<tr>
<td>Poor survival outcome (death)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor volume, cm³</td>
<td>18.7</td>
<td>0.67</td>
<td>0.55</td>
</tr>
<tr>
<td>Hematoma volume, cm³</td>
<td>3.1</td>
<td>0.83</td>
<td>0.64</td>
</tr>
<tr>
<td>Ratio of hematoma/tumor volume, %</td>
<td>19.3</td>
<td>0.83</td>
<td>0.82</td>
</tr>
</tbody>
</table>

**p < 0.05, **p < 0.01.

AUC = area under receiver operating characteristic curve; mRS = modified Rankin scale.
intracranial pressure (IICP) and obstructive hydrocephalus.13 The larger the hematoma volume, the greater its effect on brain parenchyma and acute IICP. Repeated tumor bleeding might also enlarge hematoma volume and aggravate brain damage. Another possible mechanism affecting the outcome of patients with TH is injury secondary to hematoma-related inflammatory changes. Free radicals and cytokines involved in this inflammatory process can injure the brain tissue adjacent to hematomas.14 We suggested that these patients should receive aggressive treatments, such as surgical intervention or irradiation as soon as possible. The peak incidence of PIGCT is around 10 to 12 years of age.15 In our studies, the mean age was 11.1 years. The previously reported male predominance of GCTs16 was also observed here. In our studies, TH was found in tumors located in pineal gland, suprasellar region, and basal ganglia. Hence, there is no age, sex, or tumor location prediction for spontaneous TH. That said, some clinical features of PIGCT with HT were clearly different from those of without TH (Table 1). For example, diabetes insipidus was only noted in two patients (11.8%) of this study.

Although there was no statistical significance ($p = 0.102$), we found that intracystic hematomas were smaller than solid hematomas. We hypothesize that intracystic hematomas are structurally confined, limiting their adjacent extension and size expansion. These confined intracystic hematomas might resolve with tumor control by irradiation. In contrast, vascular hematomas within solid tumors have no defined cystic wall barrier. This intratumoral hematoma may expand even beyond the tumor surface and have a significant mass effect possibly justifying early surgical intervention.

The limitations of this retrospective study included small number of patients, diverse MR patterns of TH, and lack of a single therapeutic strategy. These limitations made statistical analysis difficult. We suggest a prospective, multicenter study to evaluate more cases. A study using comprehensive imaging techniques, such as MR perfusion imaging, may be beneficial in evaluating the disease process before and after therapy.

In conclusion, our study demonstrated that (1) functional and survival outcomes were significantly worse for patients with larger HTVRs than those with smaller ones; (2) tumors with hematomas $>5.6 \text{ cm}^3$ or mean HTVR $>16.8\%$ increased risk of poor functional outcome (mRS $\geq 3$); and (3) mean HTVR $>19.3\%$ predicted poor survival outcome after treatment. In PIGCT patients with TH, early and aggressive treatment (such as radiotherapy) is recommended to improve the clinical outcomes of those with large hematoma volume and large HTVR.

ACKNOWLEDGMENTS

This study was supported by Taipei Veterans General Hospital (V106C-197) and Ministry of Science and Technology (105-2314-B-075-027-MY2).

We also thank Dr. Z. Sean Juo for English editing of this manuscript.

REFERENCES


Predictors of subsequent pregnancy in women who underwent laparoscopic cornuostomy or laparoscopic wedge resection for interstitial pregnancy

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Abstract

Background: The ideal surgical procedure for interstitial pregnancy remains undetermined. The aim of this study was to assess whether surgical method is a factor in predicting subsequent pregnancy in women with interstitial pregnancy who underwent laparoscopic cornuostomy or laparoscopic wedge resection.

Methods: Medical records of all women with interstitial pregnancy who underwent laparoscopic cornuostomy or laparoscopic wedge resection between March 2008 and October 2017 in a medical center were reviewed. Cox regression analysis was performed to identify factors predicting subsequent pregnancy.

Results: Forty patients underwent laparoscopic cornuostomy (n = 14) or laparoscopic wedge resection (n = 26) for the treatment of interstitial pregnancy. Twelve women become pregnant during follow-up. Laparoscopic cornuostomy was associated with a shorter operation time (coefficient = −19.1 minutes, 95% CI = −36.9 to −1.3 minutes, p = 0.04, multivariable analysis) than that of laparoscopic wedge resection. Furthermore, laparoscopic cornuostomy (hazard ratio = 6.3, p = 0.03), parity (hazard ratio = 0.18, p = 0.008), and preoperative rupture of the cornus (hazard ratio = 13.3, p = 0.005) were independent predictors of subsequent pregnancy.

Conclusion: Laparoscopic cornuostomy was associated with a higher probability of subsequent pregnancy and a shorter operation time. Thus, compared with laparoscopic wedge resection, laparoscopic cornuostomy might be a better surgical procedure for women with interstitial pregnancy, particularly for women who wish to become pregnant later. However, because of the retrospective nature and small sample size of this study, some well-defined/designed prospective studies including more patients are needed to verify our results.

Keywords: Cornus; Interstitial pregnancy; Laparoscopy

1. INTRODUCTION

Historically, interstitial pregnancy was treated by local methotrexate injection,¹² laparotomic resection, or hysterectomy.³ Recently, laparoscopic cornuostomy (LC) or laparoscopic wedge resection (LWR) have been used to treat women with interstitial pregnancy.⁴,⁵ However, only Lee et al.⁶ reported a between-group comparison of LC and LWR and concluded that LC may reduce the surgical time and that the incidence of persistent interstitial pregnancy is the same for both procedures. Nonetheless, to the best of our knowledge, no research has yet investigated which laparoscopic surgical procedure is better for women with interstitial pregnancy, particularly for those who desire a future pregnancy. Thus, the aim of this study was to elucidate whether LC is better than LWR for women with interstitial pregnancy, particularly for those with a desire for a future pregnancy.

2. METHODS

Medical records of all women who underwent LC or LWR for interstitial pregnancy between March 2008 and October 2017 in the Department of Obstetrics & Gynecology of a medical center were reviewed. The Research Ethics Committee of the hospital approved this study. The diagnosis of interstitial pregnancy was defined by the intraoperative identification of the pregnancy as an asymmetric bulge in one of the uterine angles lateral to the insertion of the round ligament.¹⁰

Baseline characteristics, perioperative data, and follow-up outcomes were compared between the LC and LWR groups. Because the purpose of this study was to elucidate whether LC has a better impact on subsequent pregnancy, women who had a heterotopic pregnancy found during the operation and preserved her intrauterine pregnancy during surgery, who had a documented later pregnancy by artificial reproductive technology, or
who had ever undergone contralateral salpingectomy or concomitant tubal sterilization were excluded from this study. Only those women with an intact contralateral tube after surgery were included. There were nine surgeons who performed LC or LWR in this study. The choice of operative method (i.e., LC or LWR) was based on each surgeon’s preference.

LC was usually performed as follows. Under laparoscopic inspection, the swollen cornus was identified and a linear incision was made to open the cornus, usually after diluted vasopressin local injection. If the cornus ruptured preoperatively, a linear extension was made along the site of rupture. The gestational products were removed using forceps and a suction tube when possible. After the above procedures, ooze was usually found in the implantation site. Active vascular bleeding was usually treated with electrocauterization. Then, the edges of the cornuostomy were approximated with 1-0 Vicryl (Ethicon Inc., Somerville, NJ) or V-Loc (Covidien, Mansfield, MA) sutures, and the surgeon would take care to keep the patency of the interstitial part of the fallopian tube during suturing. After suturing, ooze and related hematoma would be confined within the interstitial space, generating an increase in the intraluminal pressure. The increased intraluminal pressure would be helpful for hemostasis. An abdominal drain was usually placed to monitor any delayed postoperative hemorrhage.

LWR was usually performed as follows. Under laparoscopic inspection, the swollen cornus was identified and resected with monopolar scissors or hooks, usually after diluted vasopressin local injection and occasionally accompanied by salpingectomy, followed by approximation of the remaining myometrial defect with 1-0 Vicryl or V-Loc sutures.

The operation time was calculated from the point of skin incision to the end of the skin wound closure. Blood loss was estimated by calculating the difference between the volumes of aspirated and irrigated fluid. Complications were defined as events requiring active treatment, such as conversion to laparotomy or further laparoscopic surgery for persistent interstitial pregnancy. The surgery-pregnancy interval was calculated from the date of operation to the date of the last menstrual period that documented pregnancy or last follow-up.

The STATA software program (version 11.0; Stata Corp, College Station, TX) was used for statistical analyses. Chi-square test, Wilcoxon rank-sum test, Fisher’s exact test or multivariable linear regression analysis were used, as appropriate. Pregnancy probability was estimated using the Kaplan-Meier method. Multivariable backward stepwise Cox regression analysis was performed using all the variables in the univariate analysis. A p value of <0.05 was considered statistically significant.

### 3. RESULTS

After excluding four cases, forty patients underwent LC (n = 14) or LWR (n = 26) for interstitial pregnancy (Figure 1). The baseline characteristics of the patients are listed in Table 1. Nine gynecologic surgeons performed these surgeries. The decision to perform LC or LWR was based on each surgeon’s preference. Among the nine surgeons, five surgeons performed LWR only (n = 22), two surgeons performed LC only (n = 10), and only two surgeons performed both LC and LWR methods (n = 8). Except for body mass index, operation time and blood loss, there were no significant differences in the clinical parameters between the groups. One
A patient in the LC group who received an adjuvant methotrexate injection was a case of gestational trophoblastic neoplasia in the cornus that was confirmed after pathologic examination of the surgical specimen. Subsequent pregnancy probability of the whole population and both groups is shown in Figure 2A, B.

Univariate and multivariable backward stepwise Cox proportional hazards regression analyses were performed to predict future pregnancy after surgery for interstitial pregnancy (Tables 2 and 3). Use of the LC method (hazard ratio = 6.3, p = 0.03), parity (hazard ratio = 0.18, p = 0.008), and preoperative rupture of the cornus (hazard ratio = 13.3, p = 0.005) was independent predictors of future pregnancy.

Among eight neonates who were delivered in our hospital, five were delivered by normal spontaneous vaginal delivery and three were delivered by cesarean section. There were no postoperative, perinatal, or obstetrical complications in either group during follow-up.

Operation time was shorter in the LC group than in the LWR group (Table 1). Multivariable backward stepwise regression analysis confirmed that the LC method was the only predictor of operation time (coefficient = −19.1 minutes, 95% CI = −36.9 to −1.3 minutes, p = 0.04).

The amount of blood loss was higher in the LWR group than in the LC group (Table 1). However, multivariable backward stepwise linear regression analysis revealed that preoperative rupture of the cornus was the only predictor (coefficient = 1278 mL, 95% CI = 748–1807 mL, p < 0.001).

4. DISCUSSION

In this study, we found that LC was a significant predictor (hazard ratio = 6.3) of future pregnancy. Watanabe et al. reported that 8 of the 13 women who underwent LC experienced a spontaneous intrauterine pregnancy subsequent to surgery. Ng et al. reported that 18 of 52 (34%) women became pregnant after LC or LWR. Liao et al. reported that 71.4% of the patients who underwent laparotomic wedge resection were able to become pregnant later. However, we did not find any study that has compared the subsequent pregnancy rates between the LC and LWR methods for treating interstitial pregnancy in women who desire subsequent pregnancy. Thus, our result suggests that compared with LWR, LC may be a better procedure for treating patients with interstitial pregnancy who desire a subsequent pregnancy.

In this study, preoperative rupture of the cornus was a predictor of subsequent pregnancy (odds ratio = 13.3). No previous studies have mentioned the relationship between preoperative rupture of the cornus and subsequent pregnancy in women who have undergone surgery for interstitial pregnancy. We speculate that preoperative rupture of the cornus may be associated with more prominent cornual engorgement, and it is easier to obtain adequate cornuostomy wound edges to reconstruct a patent tubal lumen in the interstitial segment after enucleation of the gestational products. In cases of preoperative rupture of the cornus, it should be easier to enter the tubal lumen of the interstitial segment.

### Table 1

| Variables                        | Cornuostomy (n = 14) | Wedge resection (n = 26) | *p*
|----------------------------------|----------------------|--------------------------|------
| Age, y                           | 32.8 ± 5.9           | 33.0 ± 5.5               | 0.98 |
| Parity                           | 0.7 ± 0.7            | 1.0 ± 0.9                | 0.38 |
| Body mass index, kg/m²           | 21.6 ± 3.8           | 23.8 ± 4.0               | 0.02 |
| Gestational age, wk              | 7.2 ± 1.1            | 7.4 ± 2.4                | 0.54 |
| Gestational mass size, cm        | 2.3 ± 0.8            | 3.3 ± 1.5                | 0.16 |
| Baseline β-hCG, mIU/mL           | 13472 ± 15396        | 20222 ± 31830            | 0.95 |
| Right side of pregnancy         | 6 (43)               | 15 (58)                  | 0.37 |
| Preoperative rupture of the cornus | 6 (43)              | 16 (62)                  | 0.26 |
| Operation time, min             | 64.8 ± 21.7          | 83.6 ± 28.7              | 0.009|
| Blood loss, mL                   | 519 ± 1001           | 1192 ± 995               | 0.002|
| Pregnancy during follow-up       | 5 (36)               | 7 (27)                   | 0.72 |
| Adjuvant methotrexate injection  | 3 (21)               | 3 (12)                   | 0.65 |
| Median surgery-pregnancy interval, wk | 61.9 (5, -)     | 313.6 (17.6, -)          | 0.42 |
| Mean follow-up interval, wk      | 115.9 ± 124.4        | 102.5 ± 145.7            | 0.48 |

Data are expressed as the mean ± SD or number (%).

*By Wilcoxon rank-sum test, chi-squared test, Fisher’s exact test, or log-rank test.

hCG = human chorionic gonadotropin.
Table 2
Comparisons between the pregnancy and nonpregnancy groups and univariate Cox proportional hazards regression analyses for predicting subsequent pregnancy after surgery for interstitial pregnancy (n = 40)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pregnancy (n = 12)</th>
<th>Nonpregnancy (n = 28)</th>
<th>p</th>
<th>Values</th>
<th>Univariate hazard ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cornuostomy</td>
<td>5 (36)</td>
<td>9 (64)</td>
<td>0.56</td>
<td>14 (35)</td>
<td>1.61 (0.50–5.22)</td>
<td>0.42</td>
</tr>
<tr>
<td>Wedge resection</td>
<td>7 (27)</td>
<td>19 (73)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>31.6 ± 3.0</td>
<td>33.5 ± 6.3</td>
<td>0.48</td>
<td>33.0 ± 5.5</td>
<td>0.89 (0.80–0.99)</td>
<td>0.04</td>
</tr>
<tr>
<td>Parity</td>
<td>0.5 ± 0.7</td>
<td>1.1 ± 0.9</td>
<td>0.04</td>
<td>0.9 ± 0.8</td>
<td>0.42 (0.18–0.95)</td>
<td>0.04</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>21.7 ± 4.7</td>
<td>23.6 ± 4.0</td>
<td>0.04</td>
<td>23.0 ± 4.0</td>
<td>1.01 (0.79–1.29)</td>
<td>0.94</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>7.8 ± 1.8</td>
<td>7.3 ± 2.2</td>
<td>0.63</td>
<td>7.3 ± 2.0</td>
<td>1.06 (0.79–1.41)</td>
<td>0.70</td>
</tr>
<tr>
<td>Gestational mass size, cm</td>
<td>2.7 ± 0.9</td>
<td>2.9 ± 1.5</td>
<td>0.79</td>
<td>2.9 ± 1.3</td>
<td>0.63 (0.30–1.32)</td>
<td>0.22</td>
</tr>
<tr>
<td>Baseline hCG, mIU/mL</td>
<td>15771 ± 16805</td>
<td>18535 ± 30320</td>
<td>0.69</td>
<td>17735 ± 26906</td>
<td>1.000 (1.000–1.000)</td>
<td>0.39</td>
</tr>
<tr>
<td>Right side of pregnancy</td>
<td>7 (33)</td>
<td>14 (67)</td>
<td>0.63</td>
<td>21 (53)</td>
<td>1.26 (0.40–3.95)</td>
<td>0.69</td>
</tr>
<tr>
<td>Preoperative rupture of cornus</td>
<td>7 (32)</td>
<td>15 (68)</td>
<td>0.78</td>
<td>22 (55)</td>
<td>2.29 (0.72–7.29)</td>
<td>0.36</td>
</tr>
<tr>
<td>Operation time, min</td>
<td>73.3 ± 22.8</td>
<td>78.8 ± 29.9</td>
<td>0.71</td>
<td>77.2 ± 27.7</td>
<td>1.004 (0.979–1.029)</td>
<td>0.98</td>
</tr>
<tr>
<td>Blood loss, mL</td>
<td>1151 ± 1315</td>
<td>874 ± 908</td>
<td>0.76</td>
<td>967 ± 1037</td>
<td>1.001 (1.000–1.001)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SD, number (%), or hazard ratio (95% CI).

*Multivariable backward stepwise Cox proportional hazards regression analyses was performed using all variables in the univariate analysis of Table 2. Herein, we do not show the data without statistical significance.

Table 3
Multivariable backward stepwise Cox proportional hazards regression analyses for predicting subsequent pregnancy after surgery for interstitial pregnancy (n = 40)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Multivariable hazard ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornuostomy method</td>
<td>6.3 (1.2–33.9)</td>
<td>0.03</td>
</tr>
<tr>
<td>Parity</td>
<td>0.18 (0.05–0.63)</td>
<td>0.008</td>
</tr>
<tr>
<td>Preoperative rupture of the cornus</td>
<td>13.3 (2.2–81.0)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Values are expressed as the hazard ratio (95% CI).

Parity was another negative predictor of subsequent pregnancy (odds ratio = 0.18). In the era of low birth rates, it is not difficult to imagine that increased parity is associated with a low rate of subsequent pregnancy. In addition, among the nine surgeons in this study, five surgeons performed LWR only (n = 22), two surgeons performed LC only (n = 10), and only two surgeons performed both LC and LWR methods (n = 8). Thus, parity is less likely to be a major consideration for LWR in this study.

LWR was reported to be particularly useful for interstitial pregnancy >4 cm in diameter.12,13 However, we did not use the above criteria for the selection of surgical method for interstitial pregnancy. In this study, the LC group was associated with a shorter operation time (coefficient = −19.1 minutes, 95% CI = −36.9 to −1.3 minutes, p = 0.04) than that of the LWR group. Lee et al.9 also reported that the operation time of LC CI = −36.9 to −1.3 minutes, a shorter operation time (coefficient = −19.1 minutes, 95% CI = −36.9 to −1.3 minutes, p = 0.04) than that of the LWR group. Lee et al.9 also reported that the operation time of LC might be related to unbalanced sample size, as was observed in our study.

In conclusion, LC was associated with higher subsequent pregnancy probability and shorter operation time than that of LWR. Thus, LC might be a better surgical procedure for women with interstitial pregnancy, particularly for women who wish to become pregnant later. However, because of the retrospective nature and small sample size of this study, some well-defined/designed prospective studies including more patients are needed to verify the results.

REFERENCES
The mid-term outcome of dialysis-dependent patients undergoing primary total knee arthroplasty and total hip arthroplasty: A retrospective study

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1. INTRODUCTION

Total joint arthroplasty is an effective treatment for advanced osteoarthritis and osteonecrosis.1-3 Many dialysis-dependent patients develop osteoarthritis and osteonecrosis requiring arthroplasty surgeries.4-6 However, surgeons are more cautious with renal failure patients owing to the high comorbidity and risk of complications.6 Dialysis-dependent patients undergoing primary total knee arthroplasty (TKA) and total hip arthroplasty (THA) have been associated with higher in-hospital mortality and complication rates.7 McCleery et al. showed that renal failure and dialysis were independent risk factors for early infection and revision in primary TKA.8 Case series have been limited by small case numbers (<20 patients) and shorter follow-up time and were more focused on short-term complications and survival.5,9,10 We hypothesize that the mid-term outcome of dialysis-dependent patients who underwent primary TKA or THA will show satisfactory functional outcomes, low-implant failure rates, considerably higher complication rates, and high mortality rates.

2. METHODS

This retrospective single-center study used the database of the orthopedic department of a single medical center. We included patients who underwent primary TKA or THA between November 2004 and January 2015, with a minimum follow-up of 24 months. Thirty-four patients with 39 total knee arthroplasties were included (M:F, 33.3%:66.7%, mean age: 68.8 years, mean follow-up: 55.9 ± 28.3 months). Twenty-seven patients with 31 total hip arthroplasties were included (M:F, 22.6%:77.4%, mean age: 62.3 years, mean follow-up: 55.8 ± 23.4 months).

Results: In the total knee arthroplasty group, there were two in-hospital mortality cases (3.5%) and two cases of implant failure (5.1%). The mean Knee Society Score was 84.0 ± 20.7. In the total hip arthroplasty group, there were three cases of implant failure (9.7%). The mean Harris Hip Score was 81.1 ± 23.9. The complications we encountered for both groups were instability and infection.

Conclusion: Dialysis-dependent patients who had undergone total joint arthroplasty are associated with high mortality rate. In our experience, satisfactory mid-term results can be achieved in these patients with manageable complications and low-implant failure rates.

Keywords: Complication; Hemodialysis; Total hip arthroplasty; Total knee arthroplasty

Abstract

Background: Dialysis-dependent patients undergoing primary total knee and total hip arthroplasty have been associated with higher in-hospital mortality and complication rates. We investigated the mid-term implant survival, patient survival, and functional outcomes in these patients and reviewed our complications.

Methods: We retrospectively reviewed dialysis-dependent patients undergoing primary total knee or total hip arthroplasty in our hospital between November 2004 and January 2015, with a minimum follow-up of 24 months. Thirty-four patients with 39 total knee arthroplasties were included (M:F, 33.3%:66.7%, mean age: 68.8 years, mean follow-up: 55.9 ± 28.3 months). Twenty-seven patients with 31 total hip arthroplasties were included (M:F, 22.6%:77.4%, mean age: 62.3 years, mean follow-up: 55.8 ± 23.4 months).

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Keywords: Complication; Hemodialysis; Total hip arthroplasty; Total knee arthroplasty

1. INTRODUCTION

Total joint arthroplasty is an effective treatment for advanced osteoarthritis and osteonecrosis.1-3 Many dialysis-dependent patients develop osteoarthritis and osteonecrosis requiring arthroplasty surgeries.4-6 However, surgeons are more cautious with renal failure patients owing to the high comorbidity and risk of complications.6 Dialysis-dependent patients undergoing primary total knee arthroplasty (TKA) and total hip arthroplasty (THA) have been associated with higher in-hospital mortality rates and greater overall complication rates.7 McCleery et al. showed that renal failure and dialysis were independent risk factors for early infection and revision in primary TKA.8 Case series have reported satisfactory functional results after primary total joint arthroplasty.5,9,10 However, individual case series had been limited by small case numbers (<20 patients) and shorter follow-up time and were more focused on short-term complications and survival.5,9,10

We hypothesize that the mid-term outcome of dialysis-dependent patients who underwent primary TKA or THA will show satisfactory functional outcomes, low-implant failure rates, considerably higher complication rates, and high mortality rates.

2. METHODS

This retrospective single-center study used the database of the orthopedic department of a single medical center. We included patients who underwent primary TKA or THA between November 2004 and January 2015 and were on regular hemodialysis for at least 1 year before TKA or THA. Except for in-hospital mortality cases and patients who died within 24 months after surgery, all patients were followed for at least 24 months. Patients who were not dialysis-dependent for at least 1 year before the operation and patients who were lost to follow up within 24 months were excluded. The study was approved by the Institutional Review Board.

For the TKA group, we identified 49 patients who underwent a total of 57 primary TKA surgeries. Fifteen patients who were lost to follow up within 24 months were excluded from our...
study. Thus, we included 34 patients with a total of 39 TKA surgeries, of whom 13 were men (33.3%) and 26 were women (66.7%). The average age at surgery was 68.8 ± 9.0 years old (range: 51–90). The average follow-up time was 55.9 ± 28.3 months (range: 24–128, N = 39). As for the THA group, we also identified 30 patients who underwent a total of 34 primary THA surgeries. Three patients were lost to follow up within 24 months and were excluded. Thus, 27 patients with a total of 31 THA surgeries were included. Of these, seven were men (22.6%) and 24 were women (77.4%). The average follow-up time was 55.8 ± 23.4 months (range: 24–98, N = 31). The patient demographics are shown in Table 1.

2.1. Surgery and postoperative follow-up

All surgeries were elective. Careful history-taking was performed at the clinic. Individual examinations and consultations with specialists were arranged for patients with additional cardiovascular risks or underlying diseases of other organ systems. Consultation with nephrologists was arranged for every patient on admission to arrange dialysis plans during hospitalization. Patients with active infection or unstable hemodynamic conditions were precluded from surgery.

All patients underwent minimally invasive mid-vastus approach for cemented TKA procedures. Prior literature has reported conflicting results for the use of antibiotic-loaded cement in primary total joint arthroplasty, with some demonstrating decreased deep infection rates and some showing no significant difference compared with controls. In our series, we routinely used vancomycin-loaded cement for TKA owing to the high infection rate. The regimen is 1g of Vancomycin in 40g of bone cement. Modified Hardinge approach or posterolateral approach was performed for all cementless THA procedures. All patients who had undergone TKA were allowed immediate full weight-bearing without restrictions, with walking aids if necessary. Weight-bearing protocols following THA depended on bone quality and quality of the press-fit mechanism. The blood transfusion strategy was different for each operating surgeon. As a general principle, when the preoperative hemoglobin is <8mg/dl or when substantial blood loss occurs during the operation, blood transfusion with packed red blood cells is performed during the operation. Hemoglobin is routinely checked on the first postoperative day. If the hemoglobin is <8mg/dl or there is a substantial drop associated with anemic symptoms, blood transfusion is performed. In our series, the transfusion rate in the THA group was 66.7%, and 78.6% in the TKA group.

After discharge from the hospital, patients were regularly followed at our outpatient clinic at postoperative 2 weeks, 2 months, 12 months, and then annually, for wound assessment, radiographic evaluation, and functional evaluation. Additional visits for various reasons were also recorded.

2.2. Radiographic evaluation

Plain films were retrospectively reviewed by an attending doctor who had not participated in any of the surgery, and was blinded to the clinical results of the patients. All plain films were reviewed for episodes of dislocation, implant alignment, evidence of loosening, subsidence, implant wear, and periprosthetic fracture.

2.3. Implant failure

Implant failure was defined as persistent or recurrent instability with more than two episodes of recorded dislocation, implant loosening or wear requiring revision arthroplasty, or infection resulting in two-stage exchange arthroplasty. Other complications were also recorded.

2.4. Functional evaluation

The data for the functional evaluation were obtained at the last visit. We used Knee Society Score (KSS), Knee Society Score-Function (KSS-F), and the Western Ontario and McMaster Universities Arthritis Index (WOMAC) to evaluate knee function in the TKA group. We used Harris Hip Score (HHS) to evaluate hip function in the THA group.

2.5. Data analysis

Kaplan-Meier survival analysis was used to determine implant and patient survival. Our primary endpoint was implant failure because of aseptic loosening, infection, or instability that required additional surgery. The endpoint for patient survival was any-cause mortality following total joint arthroplasty. SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used for all analyses.

3. RESULTS

3.1. Total knee arthroplasty

The 49 preexclusion patients (57 TKA surgeries) included two in-hospital mortality cases and two patients died within 24 months after surgery. The calculated overall in-hospital mortality rate was 3.5% (2/57). Causes of the two in-hospital mortality cases were pneumonia and subdural effusion after a fall during hospitalization. The other deaths included a patient with

### Table 1

<table>
<thead>
<tr>
<th>Patient demographics</th>
<th>TKA</th>
<th>THA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient number/surgery</td>
<td>34/39</td>
<td>27/31</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13 (33.3%)</td>
<td>7 (22.6%)</td>
</tr>
<tr>
<td>Female</td>
<td>26 (66.7%)</td>
<td>24 (77.4%)</td>
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<tr>
<td>Mean age at operation (range)</td>
<td>68.8 ± 9.0 (51–90)</td>
<td>62.3 ± 16.7 (28–83)</td>
</tr>
<tr>
<td>Comorbidity</td>
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<tr>
<td>HTN</td>
<td>51.3%</td>
<td>29%</td>
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<tr>
<td>DM</td>
<td>30.8%</td>
<td>12.9%</td>
</tr>
<tr>
<td>CAD</td>
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<td>19.4%</td>
</tr>
<tr>
<td>Cancer</td>
<td>15.4%</td>
<td>6.5%</td>
</tr>
<tr>
<td>Stroke</td>
<td>10.3%</td>
<td>0%</td>
</tr>
<tr>
<td>SLE</td>
<td>0%</td>
<td>19.4%</td>
</tr>
<tr>
<td>HBV, HCV</td>
<td>5.1%</td>
<td>9.7%</td>
</tr>
<tr>
<td>Other heart diseasea</td>
<td>5.1%</td>
<td>19.4%</td>
</tr>
<tr>
<td>Thyroid disease</td>
<td>7.7%</td>
<td>0%</td>
</tr>
<tr>
<td>Mean f/u time (range)</td>
<td>55.9 ± 28.3 months (24–128)</td>
<td>55.8 ± 23.4 (24–98)</td>
</tr>
</tbody>
</table>

*aOther heart disease includes atrial fibrillation, heart failure, and valvular heart disease.

CAD = coronary artery disease; DM = diabetes mellitus; HBV = hepatitis B; HCV = hepatitis C; HTN = hypertension; SLE = systemic lupus erythematosus.

### Table 2

<table>
<thead>
<tr>
<th>Complications for total knee arthroplasty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient no.</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>25</td>
</tr>
</tbody>
</table>

CAD = coronary artery disease; DM = diabetes mellitus; Prostate CA = prostate cancer.
an arterio-venous fistula infection 4 months after surgery and a patient who developed pneumonia 20 months after surgery. There were three cases of surgical complications (overall complication rate: 7.7%), which include a patient with persistent dislocation who remained wheelchair-bound, a patient with instability requiring casting, and a patient with periprosthetic joint infection requiring open arthroscopy at postoperative 20 months. The summary is shown in Table 2. The first two cases were regarded as implant failures (overall implant failure rate: 5.1%). The third patient received 8 more weeks of oral antibiotics and no further surgery was needed. He was followed for a total of 74 months with a final KSS of 95. The implant survival mean was 120.8 ± 5.0 months, while the implant survival rates for the first and fifth years were 94.3% and 94.3%, respectively. The mean patient survival time was 91.3 ± 9.1 months. The patient survival rates in the first, second, and fifth years were 90.3%, 87.5%, and 69.7%, respectively. The survival curve is shown in Figure 1.

The mean KSS score was 84.0 ± 20.7 (range: 0-95), the mean KSS-F was 56.3 ± 35.1 (range: 0-90), and the mean WOMAC score was 78.4 ± 20.7 (range: 15.9-96).

3.2. Total hip arthroplasty

The overall in-hospital mortality rate was 0%. There were four cases of complications (overall complication rate: 12.9%). One patient developed periprosthetic joint infection and underwent debridement surgery followed by implant resection at 24 months and revision arthroplasty at 28 months after the initial operation. The patient was followed for a total of 94 months, and had a good HHS score on the final visit. Two patients had recurrent dislocations, with the first episode occurring at postoperative 1 and 37 months, respectively. The other patient whose intraoperative wound culture yielded positive bacterial growth underwent oral antibiotic treatment for 6 months, and no further debridement surgery or revision arthroplasty was needed. She was followed up for 35 months with an excellent HHS score on the final visit. The summary is shown in Table 3. The first three cases were regarded as implant failures (overall implant failure rate: 9.7%). The implant survival mean was 89.9 ± 4.5 months, while the implant survival rates in the first, second, and fifth years were 96.8%, 93.5%, and 89.3%, respectively. The mean patient survival time was 79.8 ± 5.5 months and the median patient survival was 94.0 ± 7.8 months. The patient survival rates in the first, second, and fifth years were 100%, 96.8%, and 67%, respectively. The survival curve is shown in Figure 2.

The mean HHS score was 81.1 ± 23.9 (range: 22.5-98), with 70.6% of the patients having a good to excellent result.

4. DISCUSSION

Our study focused on mid-term implant survival, patient survival, and functional outcome. Our mean follow-up time exceeded 4 years for both groups. The mean age at TKA and THA was 68.8 and 62.3 years, respectively, which is comparable to that in prior literature. Kaplan-Meier survival analysis showed a high implant survival rate, while the mortality rate was high. The general functional results were satisfactory, despite some complications.

Ponnusamy et al. found a higher in-hospital mortality rate in dialysis-dependent patients undergoing primary TKA and THA, with rates of 9.2% and 1.88%, respectively. The overall complication rates were also higher, with 12.48% and 9.98% for TKA and THA, respectively. The registry study conducted by Miric et al. concluded that patients with chronic renal disease undergoing primary TKA and THA surgeries had significantly higher incidence of deep and superficial surgical site infection, higher rate of 90-day and any-time mortality, and 90-day readmission rate, compared with non-chronic renal disease patients. In our study, there were two in-hospital mortality cases in the TKA group (3.5%) and none in the THA group. The rate is high but could be biased by our relatively small sample size. Our TKA group included three complications. One patient developed dislocation at postoperative two months and remained dislocated after two episodes of close reduction, being wheelchair bound since. The patient was a case of old septic arthritis with substantial bone loss over the lateral posterior femoral condyle. No evidence of active infection was noted during the operation; however, the bone loss may have misled the surgeon in determining femur rotation, resulting in a large mid-flexion gap. This complication could be mostly attributed to poor surgical technique. The preoperative and postoperative plain film of this patient is shown in Figure 3. The other patient developed instability at 1 month after the operation requiring casting with poor final functional outcome. Instability is not a commonly discussed complication after TKA in the comparison of dialysis and nondialysis patients. We suspect this could be due to generalized muscle atrophy, functional decline, and poor surgical technique. These two complications were categorized as implant failure owing to a lack of function and persistent pain. McCleery et al. stated that renal failure is an independent risk factor for early infection and that renal dialysis is an
independent risk factor for early revision. In our study, there was one case of late infection and no cases that required revision surgery.

Our THA group included four complications, including two cases of infection, one resulting in joint resection and revision arthroplasty (overall infection rate: 6.5%, revision rate: 3.3%), two cases of recurrent dislocation (overall dislocation rate: 6.5%), and no case of aseptic loosening. In our study group, cementless THA was routinely used. Traditionally, cemented THA is more advocated in dialysis-dependent patients owing to considerations for the poor bone quality. However, past literature has shown conflicting results, with high loosening rates for cemented implants.\textsuperscript{16–18} In contrast, more recent series with relatively shorter follow-up time have reported good outcomes with cementless THA.\textsuperscript{19,20} At present, it may not be possible to draw a conclusion as to which fixation method is more suitable for this patient group. Nevertheless, no implant loosening was observed in our retrospective series. Lieu et al.\textsuperscript{10} found a revision rate of 2.6% at 12 months and 16.3% over the study period, and a dislocation rate of 6.5% for hemodialysis patients after THA. Our dislocation rate was comparable, while our revision rate was lower. According to past literature, TKA and THA are associated with an increased risk of deep infection, early revision, and mortality. In our experience, the cases of deep infection have been successfully managed with antibiotics and surgery.

We examined the patient survival curve and implant survival curve. In the TKA group, 28.2% patients expired during the course of follow up. All implant failure cases occurred within the first year postoperatively, resulting in a high implant survival rate. In the THA group, 32.3% patients expired during the course of follow up. The implant survival rates in the first, second, and fifth years were 96.8%, 95.5%, and 89.3%, respectively, showing a consistent decline, but still remained relatively high. Causes of late complications requiring revision surgery such as implant wear and loosening were not observed during follow up. It is arguable that these complications may take longer to develop. However, the low physical demand of dialysis-dependent patients may also contribute to the low-implant wear rate. Moreover, poor bone quality associated with dialysis-dependent patients did not translate into increased implant loosening. Nevertheless, late infections can pose a threat to implant survival, and should be managed with extra caution.

In a series of 15 TKA surgeries, Lizaur-Utrilla et al. reported a KSS score of 87.9, KSS-F of 81.3, and WOMAC of 78.4,\textsuperscript{3} while Chen et al. reported a KSS score of 79 and KSS-F of 81 in a series of 18 TKA surgeries.\textsuperscript{2} Our KSS and WOMAC results were comparable; however, the KSS-F score (mean: 36.3) was significantly lower. In a review article, Lieu et al.\textsuperscript{10} reported an HHS of 65.6 after the THA surgery, with 45.9% of the patients receiving a good to excellent result. Our average HHS score was higher, with a greater percentage of patients receiving a good or excellent result. The majority of our patients do benefit from the operation in terms of functional improvement and pain relief. However, the KSS-F in our TKA patients

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**Fig. 2** Patient survival and implant survival in total hip arthroplasty.

**Fig. 3** This is a 51-year-old female patient with history of old septic arthritis of the right knee. A, Preoperative AP and lateral plain film shows severe bone loss over the lateral condyle; B, Postoperative plain film; C, Postoperative 3-month plain film shows persistent dislocation of the joint.
was significantly lower than in the aforementioned studies. Moreover, three patients in the THA group had a poor HHS score.

Upon reviewing the questionnaire, we found nine patients who scored 40 or less in the KSS-F section. Six patients had no or minimal pain after the operation and could ambulate, but were household-bound due to general health conditions, thus resulting in a low score. The other three patients had moderate to severe pain and could not ambulate at all. One of these was the patient with persistent dislocation who we mentioned earlier. The other two patients had a history of coronary artery disease and stroke and had poor ambulatory function. As this particular group of patients had high comorbidity rates and low physical demand, a low KSS-F score may not necessarily translate to a low satisfaction rate for surgery or quality of life.

The strength of our study lies in the larger case number compared to that in past series and longer mean follow-up times. Moreover, we were the first to tentatively provide a survival curve for this patient group. However, this was a retrospective study. Although we included all patients in our hospital matching these criteria, those who were lost to follow up within 2 years were excluded, which could bias our results. In addition, we did not have preoperative functional scores, hindering us from providing data on the relative improvement after surgery. Fortunately, most of our patient demographics and study results were comparable to those of past literature. Future studies with larger case numbers and longer follow-up times are needed to better understand the long-term functional outcomes, quality of life, and complications in this challenging patient group.

In conclusion, we present our case series of dialysis-dependent patients undergoing primary TKA and THA surgery. The complications encountered in TKA were persistent dislocation, instability, and infection; those in the THA group included recurrent dislocation and infection. The infection cases were successfully managed with antibiotics and surgery. There were no cases of loosening or severe implant wear. The implant survival rate was high in both groups and the functional results were generally good. Considering the relatively low physical demand and high mortality rate, we believe that total joint arthroplasty can achieve satisfactory mid-term results with manageable complications and low-implant failure rates in dialysis-dependent patients.

REFERENCES
The impact on outcomes by using thiotepa in tandem transplant for pediatric high-risk embryonal brain tumors

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Abstract

\textbf{Background:} Despite aggressive treatment including surgery, radiotherapy, and adjuvant chemotherapy, the outcome of pediatric high-risk embryonal brain tumors remains poor; especially in young children, in whom early radiotherapy inevitably brings significant long-term morbidities. Single or tandem autologous stem cell transplant has been reported to improve outcomes; but optimal use is not well defined.

\textbf{Methods:} Pediatric patients with high-risk embryonal brain tumors who underwent tandem transplant as consolidation from August 2011 to December 2017 were included. We performed a retrospective chart review and analyzed the outcomes to identify possible prognostic factors.

\textbf{Results:} Eleven pediatric patients with high-risk embryonal brain tumors were enrolled. They received double or triple autologous transplant at complete response in 5 patients and at partial response in 6 for a total of 24 transplants. There were five atypical teratoid rhabdoid tumors, four medulloblastoma, one primitive neuroectodermal tumors, and one pineoblastoma. Median age at diagnosis was 1.8 years (range, 0.6-11.2 years) and at transplant was 2.2 years (range, 1.2-11.9 years). Thiotepa-based regimens were used in 13 cycles of conditioning. All patients achieved successful engraftment. No transplant-related mortality was identified. With a median follow-up of 21.2 months (range, 6.9-51.8 months), seven patients had disease progression. Disease entity and the use of one or more cycles of thiotepa-based regimen during tandem transplant had statistically significant impact on both progression-free survival and overall survival.

\textbf{Conclusion:} With successful engraftment and manageable toxicity, tandem transplant in pediatric patients with high-risk embryonal brain tumor is feasible and safe. Patients receiving tandem transplant with one or more cycles of thiotepa-based regimen might have better outcome than those without. In combination with salvage radiotherapy, a favorable 2-year overall survival could be achieved in the majority of patients.

\textbf{Keywords:} Pediatric embryonal tumors; Tandem transplant; Thiotepa-based conditioning regimen

1. INTRODUCTION

Brain tumors are the second most common cancer in childhood. Embryonal brain tumors including medulloblastomas (MB), atypical teratoid rhabdoid tumors (ATR), primitive neuroectodermal tumors (PNET), and pineoblastomas are the most frequent malignant brain tumors in children.\textsuperscript{1} Even with contemporary treatment such as surgery, radiotherapy (RT), and chemotherapy, those patients with high-risk features, including recurrent or metastatic disease, residual tumors, and children <3 years of age, still have worse prognosis.\textsuperscript{1,2} Radiotherapy has been shown to be important in the treatment for those patients with embryonal tumors, however, at the price of endocrine disorders, cognitive impairment, intellectual disability, and second malignancy; especially in young children, in whom the developing brain is at risk.\textsuperscript{1,3}

During past few decades, a series of prospective studies with the aims of effective and optimal treatment in children with embryonal tumors had been performed, taking into consideration of age range, clinical risk stratification, and molecular classification. For MB, the clinical factors for high-risk group include age <3 years old, the size of residual tumor >1.5 cm\textsuperscript{2}, initial leptomeningeal seeding according to Chang’s classification,\textsuperscript{4} and the histological phenotype of large cell/anaplastic features.\textsuperscript{5} The most updated MB classifications identified four distinct molecular subgroups: WNT, SHH, group 3, and group 4 by their genetic expression profile.\textsuperscript{6} Patients with WNT subtypes had significant better survival than those with SHH or non-SHH/WNT tumors. However, clinical high-risk factors still significantly influenced survival in both SHH and non-SHH/WNT subgroups.\textsuperscript{7} The clinical trial of treatment stratification by combining molecular
and clinical factors in newly diagnosed MBs was still ongoing (NCT01878617).

High-dose chemotherapy with autologous hematopoietic stem cell transplant (HSCT) has been used as consolidation to overcome chemotherapy resistance of tumor and the blood-brain barrier (BBB).<sup>2,3,6,11</sup> and typically it has been given after operation, with or without definite RT, and conventional chemotherapy. This therapy has been shown to improve results, not only as salvage therapy,<sup>2,11</sup> but also as up-front treatment in pediatric patients with embryonal brain tumors. Some study groups also found that RT could be safely delayed or avoided in children treated using HSCT. Different combinations in the conditioning regimen have been used, and thiopeta often has been included<sup>2,15-16</sup> for its lipophilic nature and better BBB penetration.<sup>18</sup> Less commonly, nonthiotepa-based condition regimen also has been applied.<sup>16,17</sup> The impact of different combinations in the conditioning regimen on outcome in pediatric patients with embryonal brain tumors receiving autologous stem cell transplant is still not known.

With increasing evidence of clinical benefit in pediatric patients with high-risk features receiving a single transplant, further dose intensification by using tandem transplant to safely deliver several courses of high-dose chemotherapy was revealed to improve outcomes.<sup>1,2,15</sup> and avoid or reduce RT dose without compromising survival.<sup>1,2,24</sup> In this study, we retrospectively evaluated the feasibility and efficacy of tandem transplant in children with high-risk embryonal brain tumors, while deferring RT in young children until disease has progressed.

2. METHODS

2.1. Patients

Between February 2011 and December 2017, 11 children with embryonal brain tumors receiving tandem transplant as consolidation treatment at Division of Pediatric Hematology/Oncology of Taipei Veterans General Hospital were enrolled, according to the consensus of pediatric neuro-oncology multidisciplinary team, which consists of hematologic oncologists, neuro-oncologists, neurosurgeons, radiation oncologists, and neuroradiologists. All patients and parents or legal guardians provided informed consent before chemotherapy treatment. The cutoff point for data analyses was April 2018.

2.2. Treatment before tandem transplant

All patients underwent an evaluation of extent of disease, including whole brain and spine magnetic resonance imaging at initial diagnosis and every 3 months during treatment. Maximal surgical resection of the primary lesion was performed with attention to preservation of neurological function. For children with MB with high-risk features, eg residual tumor >1.5 cm<sup>2</sup>, 3 years of age, leptomeningeal seeding, or recurrence, and for children of any age with embryonal brain tumors other than MB, conventional chemotherapy with ifosfamide (2400 mg/m<sup>2</sup>/day, days 1-3), cisplatin (90 mg/m<sup>2</sup>/day, day 2), and etoposide (150 mg/m<sup>2</sup>/day, days 1-3) for five cycles was given, followed by tandem transplant for 2 to 3 cycles, depending on the sufficiency of peripheral blood stem cells and also the reimbursement of insurance coverage from the national health insurance system in our country. Focal or craniospinal RT was deferred in those <3 years of age unless progression or leptomeningeal seeding occurred during conventional chemotherapy. The average dose for salvage RT was 45 to 50 Gy/1.8 Gy per fraction to the primary tumor bed and 36 Gy/1.8 Gy per fraction to craniospinal axis. For patients 3 years of age without seeding, the total dose to the primary tumor bed and craniospinal axis was 50 to 56 Gy and 30 Gy, respectively.

2.3. Tandem transplant

The conditioning regimens in tandem transplant included either thiopeta-based regimen (carboplatin 500 mg/m<sup>2</sup>/day, day −8 to −6; thiopeta 300 mg/m<sup>2</sup>/day, day −5 to −3; etoposide 250 mg/m<sup>2</sup>/day, day −5 to −3) or melphalan-based regimen (cyclophosphamide 1500 mg/m<sup>2</sup>/day, day −8 to −3; melphalan 60 mg/m<sup>2</sup>/day, day −4 to −2) at about an 8-week interval to prevent toxicity from tandem transplant. Hematopoietic stem cells containing ≥2 × 10<sup>6</sup> CD34+ cells/kg were infused on day 0. During transplant, patients were isolated in a single room and received antibiotic prophylaxis including levofloxacin, micafungin, and metronidazole from day −10 until engraftment. Neutrophil engraftment is defined as the first day of absolute neutrophil count exceeding 500/μL for 3 successive days and platelet engraftment as the date of platelet count exceeding 20 000/μL without transfusion for 7 days.

2.4. Data collection

Patient characteristics, disease status before transplant, stem cell dose, times of transplant, use of a thiopeta-based regimen, engraftment, posttransplant complications, and outcome were evaluated by retrospective chart review.

2.5. Statistics

Overall survival (OS) rate was defined from the first stem cell infusion date to death or the date of the last follow-up for living patients. Progression-free survival (PFS) rate was assessed from the first stem cell infusion date to the date of progression, relapse, or death. OS and PFS were estimated by using the Kaplan-Meier analysis, and the impact of the patient-, disease-, or treatment-related factors on survival was compared using log-rank test, in which p < 0.05 was considered statistically significant.

3. RESULTS

3.1. Patient characteristics

Patient demographics, disease status, and treatment for 11 patients are listed in Table 1. Gross total or near total removal of the primary tumor was achieved in seven patients, and leptomeningeal seeding was present at initial diagnosis in three patients. Among seven patients <3 years of age at diagnosis, one received focal RT before transplant due to progression, and the other patient (with pineoblastoma) received craniospinal RT with boosting on primary tumor bed due to his age, approaching 3 years. Of the four patients with MB receiving tandem transplant, two had leptomeningeal seeding at diagnosis, one had recurrent disease, and one was <3 years old. Complete response (CR) was achieved by the end of induction chemotherapy in five patients and partial response (PR) in six.

3.2. HSCT details and complications

Nine patients received autologous HSCT twice and the others received three times. Thirteen (54%) cycles of thiopeta-based conditioning were used among a total of 24 transplants. Table 2 shows the treatment details and patient outcomes of tandem transplant. The median infused CD34+ cell dose was 4709 × 10<sup>6</sup>/kg (range, 1808-28962 × 10<sup>6</sup>/kg). Ten patients had 22 infusions of autologous peripheral blood stem cells (PBSCL), while the other one had infusions of PBSCL and bone marrow for each HSCT due to poor PBSCL mobilization, probably related to heavy treatment before PBSCL harvest. All patients achieved successful engraftment. The median days for neutrophil and platelet engraftment were 10 days (range, 8-15 days) and 18 days (range, 7-56 days), respectively.

Posttransplant complications included 22 episodes of neutropenic fever (92%), 14 cases of gastroenteritis or mucositis (58%), three instances of septicaemia or bacteremia (13%), six fungal infections (25%), four instances of upper or lower gastrointestinal bleeding (17%), two cases of hemorrhagic cystitis (8%), one cytomegaloviral viruria (4%), and one instance of skin exfoliation over skin folds (4%). No transplant-related mortality was identified.
3.3. Use of irradiation

Of the seven children <3 years of age, pretransplant craniospinal RT was avoided in six patients. One patient (no 3) with intradural and extramedullary spinal ATRT over T11-L4 with extraspinal extension received scheduled postransplant focal and craniospinal RT at the age of 2.8 years due to persistence of residual tumor after a triple transplant, but he remains progression free for 46.3+ months to date. Among the other five young children without craniospinal RT, four had progression or relapse requiring salvage RT after tandem transplant and were <24 months of age at diagnosis (three ATRT, one PNET). Only one young patient with MB who received no focal and craniospinal RT had no subsequent relapse.

3.4. Outcome

With a median follow-up of 21.2 months (range, 6.9-51.8 months) after the first HSCT, the 2-year Kaplan-Meier estimate for PFS and OS were 36 ± 15% and 80 ± 13%, respectively (Figure 1A). Seven patients had tumor progression or relapse at a median interval of 8.7 months (range, 2.9-16.1 months) and received salvage treatment, including intrathecal chemotherapy and RT. Four patients died of disease progression.

The disease entity had a statistically significant impact on 2-year PFS (Figure 1B) but not on OS. Patients with MB had 2-year PFS of 50 ± 25%, while patients with ATRT or other diseases had that of 40 ± 22% and 0%, respectively ($p = 0.02$). The 2-year OS were 100% for MB, 80 ± 18% for ATRT, and 0% for other diseases ($p = 0.10$). In addition, patients who received ≥1 cycle of thiotepa-based regimen during tandem transplant had significantly better outcome on both 2-year PFS (44 ± 17% vs 0%) (Figure 1C) and OS (100% vs 0%) than those who did not ($p < 0.001$ for PFS; $p = 0.001$ for OS). The 2-year PFS and OS rates were also higher in patients with CR before the first HSCT than in those with PR (PFS 60 ± 22% vs 17 ± 15%, OS 100% vs 63 ± 21%) (Figure 1D), which were statistically insignificant ($p = 0.16$ for PFS, $p = 0.11$ for OS).

Neither age at diagnosis or at first HSCT, interval between diagnosis to first HSCT, or use of RT before the first HSCT had statistically significant impact on outcome.

### Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (Male:Female)</td>
<td>5:6</td>
</tr>
<tr>
<td>Median age at diagnosis, y (range)</td>
<td>1.8 (0.6-11.2)</td>
</tr>
<tr>
<td>Median age at first HSCT, y (range)</td>
<td>2.2 (1.2-11.9)</td>
</tr>
<tr>
<td>Interval between diagnosis to first HSCT, m (range)</td>
<td>7.3 (4.8-20.5)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>4</td>
</tr>
<tr>
<td>ATRT</td>
<td>5</td>
</tr>
<tr>
<td>PNET</td>
<td>1</td>
</tr>
<tr>
<td>Pineoblastoma</td>
<td>1</td>
</tr>
<tr>
<td>Disease status before first HSCT</td>
<td></td>
</tr>
<tr>
<td>Complete response</td>
<td>5</td>
</tr>
<tr>
<td>Partial response</td>
<td>6</td>
</tr>
<tr>
<td>Radiotherapy before first HSCT</td>
<td></td>
</tr>
<tr>
<td>Focal alone</td>
<td>1</td>
</tr>
<tr>
<td>Focal + Craniospinal axis</td>
<td>5</td>
</tr>
<tr>
<td>No</td>
<td>5</td>
</tr>
<tr>
<td>Times of HSCT</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Conditioning regimen (n = 24)</td>
<td></td>
</tr>
<tr>
<td>Thiotepa based</td>
<td>13</td>
</tr>
<tr>
<td>Nonthiotepa based</td>
<td>11</td>
</tr>
</tbody>
</table>

**ATRT = atypical teratoid rhabdoid tumor; HSCT = hematopoietic stem cell transplant; PNET = primitive neuroectodermal tumor.**

### Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at diagnosis and first HSCT, y</th>
<th>Disease and status before first HSCT</th>
<th>Radiotherapy before first HSCT</th>
<th>Times of HSCT</th>
<th>Times of thiotepa-based regimen usage</th>
<th>PFS, mo</th>
<th>OS, mo</th>
<th>Disease status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.3/1.8</td>
<td>ATRT, PR</td>
<td>Focal</td>
<td>2</td>
<td>0</td>
<td>5.7</td>
<td>13.9</td>
<td>DOD</td>
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<tr>
<td>3</td>
<td>1.3/1.9</td>
<td>MB, CR</td>
<td>…</td>
<td>2</td>
<td>0</td>
<td>51.8+</td>
<td>51.8+</td>
<td>NED</td>
</tr>
<tr>
<td>4</td>
<td>1.8/2.2</td>
<td>Spinal ATRT, PR</td>
<td>…</td>
<td>3</td>
<td>3</td>
<td>46.3+</td>
<td>46.3+</td>
<td>NED</td>
</tr>
<tr>
<td>5</td>
<td>1.5/2.0</td>
<td>Spinal PNET, PR</td>
<td>…</td>
<td>2</td>
<td>0</td>
<td>2.9</td>
<td>6.9</td>
<td>DOD</td>
</tr>
<tr>
<td>6</td>
<td>0.6/1.2</td>
<td>ATRT, PR</td>
<td>…</td>
<td>2</td>
<td>2</td>
<td>6.7</td>
<td>28.0</td>
<td>DOD</td>
</tr>
<tr>
<td>7</td>
<td>0.9/1.5</td>
<td>ATRT, CR</td>
<td>…</td>
<td>1</td>
<td>1</td>
<td>12.0</td>
<td>21.8+</td>
<td>AWD</td>
</tr>
<tr>
<td>8</td>
<td>8.1/10.0</td>
<td>MB with seeding, CR</td>
<td>Focal + CSI</td>
<td>2</td>
<td>1</td>
<td>21.2+</td>
<td>21.2+</td>
<td>NED</td>
</tr>
<tr>
<td>9</td>
<td>7.1/7.8</td>
<td>ATRT, CR</td>
<td>Focal + CSI</td>
<td>2</td>
<td>1</td>
<td>18.2+</td>
<td>18.2+</td>
<td>NED</td>
</tr>
<tr>
<td>10</td>
<td>3.9/5.6</td>
<td>MB, 2nd PR</td>
<td>Focal + CSI</td>
<td>2</td>
<td>1</td>
<td>9.1</td>
<td>9.2+</td>
<td>AWD</td>
</tr>
<tr>
<td>11</td>
<td>2.9/3.6</td>
<td>Pineoblastoma, CR</td>
<td>Focal + CSI</td>
<td>2</td>
<td>1</td>
<td>7.2</td>
<td>8.3+</td>
<td>AWD</td>
</tr>
</tbody>
</table>

**ATRT = atypical teratoid rhabdoid tumor; AWD = alive with disease; CR = complete response; CSI = craniospinal irradiation; DOD = died of disease; HSCT = hematopoietic stem cell transplant; MB = medulloblastoma; NED = no evidence of disease; OS = overall survival; PFS = progression-free survival; PNET = primitive neuroectodermal tumor; PR = partial response.**

### 4. DISCUSSION

Our retrospective study demonstrated the feasibility of tandem transplant in pediatric patients with high-risk embryonal brain tumors with successful engraftment and manageable toxicity profile. In our study, the 2-year Kaplan-Meier estimate for PFS was 36 ± 15%, which is comparable with other contemporary studies.3,22,24,27 The relatively high 2-year OS (80 ± 13%) might be related to use of salvage RT and chemotherapy, which nevertheless did not prevent further recurrence.

For our four patients with MB, the efficacy of tandem transplant is not satisfactory with the 2-year PFS 50 ± 25%. In a study by Dufour et al,23 patients with high-risk embryonal tumors, mostly MB, were enrolled and received tandem transplant as frontline therapy. Their 3-year EFS and OS were greater, 79% and 82%, respectively, which reflected enrollment of older patients and the use of tandem transplant as frontline treatment. Sung et al20 and Gilman et al25 both investigated the feasibility of tandem transplant in MB patients with relapse and reported a 3-year EFS 29% and 25%, respectively. With the introduction of subgrouping by genetic signatures in MBs according to WHO classification in 2016,4 the outcome differences among MB subgroups may be revealed, which may also guide in optimal use of tandem transplant in patients with MB in the future.

The treatment results in five children with ATRT seem promising with 2-year PFS and OS of 40.0 ± 22% and 80 ± 18%, respectively. In the past, patients with ATRT receiving conventional therapy had a dismal prognosis with a 3-year EFS of 13% in a German HIT database during 1988-2004.24 In Head Start III using single transplant in patients with ATRT at frontline
during 2003-2009, 21% EFS was achieved at 3 years. With the advancement of tandem transplant later, induction chemotherapy, followed by tandem transplant using three cycles of thiotepa and carboplatin conditioning, was applied in children with ATRT in the study of Sick Kids with promising results. In a COG study (ACNS0333), 65 patients with ATRT of any age were treated with tandem transplant frontline and involved field RT, and the 2-year EFS was 42%.

A retrospective study by Guerra et al. of 44 pediatric patients receiving tandem transplant showed that 7 of 12 children with embryonal brain tumors receiving tandem transplant and who were <3 years of age did not relapse despite avoiding any RT, including five patients with MB and two with PNET. However, three children with ATRT who were <3 years of age relapsed at posttransplant 0.1-0.56 years and ultimately received RT. Sung et al. reported a series of 25 children <3 years of age withholding RT till 3 years of age or avoiding until relapse, in which a total of 16 patients had embryonal brain tumors. Use of any RT was abandoned until relapse/progression in five children with embryonal tumors completing double transplant, among whom only one child with MB did not have subsequent relapse. The other four children (two with ATRT, one with MB, and one with PNET) experienced progression of disease after double transplant, and three of them received salvage RT. Our data, consistent with the reports in the literatures, showed four (3 ATRT and 1 PNET) of five young children <2 years of age without craniospinal RT had progression or relapse requiring salvage RT after tandem transplant. These findings demonstrate that tandem transplant may safely delay and even avoid the use of RT in some selected cases with embryonal brain tumors, but may not prevent tumor progression/relapse, especially in children with ATRT under 3 years of age.

Fig. 1 Survival of 11 patients with embryonal brain tumors receiving tandem transplant. A, 2-year PFS and OS. B, 2-year PFS among different disease groups. C, 2-year PFS in patients receiving no cycles or ≥1 cycle of thiotepa-based regimen. D, 2-year PFS in patients achieving CR or PR before first HSCT. ATRT, atypical teratoid rhabdoid tumor; CR, complete response; HSCT, hematopoietic stem cell transplant; MB, medulloblastoma; OS, overall survival; PFS, progression-free survival; PR, partial response.
### Table 3

**Outcome of tandem transplant in pediatric patients with embryonal brain tumors**

<table>
<thead>
<tr>
<th>Study</th>
<th>Period</th>
<th>Patient inclusion (n)</th>
<th>Disease (n)</th>
<th>HSCT times</th>
<th>Conditioning regimen</th>
<th>Interval</th>
<th>PFS or EFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study</td>
<td>2011-2017</td>
<td>Fresh (10) Relapse (1)</td>
<td>ATRT (5) MB (4) Others (2)</td>
<td>2-3</td>
<td>Thio + carbo + VP16 or Mel + endoxan</td>
<td>8 wks</td>
<td>2-y PFS: 36%</td>
<td>2-y OS: 40%</td>
</tr>
<tr>
<td>ACNS0333 (COG)</td>
<td>2008-2014</td>
<td>Fresh (65)</td>
<td>ATRT (63)</td>
<td>3</td>
<td>Thio + carbo → Thio + carbo → Thio + carbo</td>
<td>4 wks</td>
<td>2-y EFS: 42%</td>
<td>2-y OS: 53%</td>
</tr>
<tr>
<td>Korea</td>
<td>2004-2012</td>
<td>Fresh (13)</td>
<td>ATRT (13)</td>
<td>2</td>
<td>Thio + carbo + VP16 → Mel + endoxan</td>
<td>12 wks</td>
<td>5-y EFS: 39%</td>
<td>5-y OS: 35%</td>
</tr>
<tr>
<td>Children Hospital of Los Angeles</td>
<td>1999-2012</td>
<td>Fresh (27) Relapse (17)</td>
<td>MB (11) PMET (11) ATRT (8) Others (14)</td>
<td>2-3</td>
<td>Thio ± others</td>
<td>3-4 wks</td>
<td>5-y EFS: 40%</td>
<td>5-y OS: 52%</td>
</tr>
<tr>
<td>Sick Kids</td>
<td>2003-2008</td>
<td>Fresh and &lt;4 y/o (8)</td>
<td>ATRT (8)</td>
<td>3</td>
<td>Thio + carbo → Thio + carbo → Thio + carbo</td>
<td>3 wks</td>
<td>4 patients are alive without evidence of tumor at a median follow-up of 52 mo</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>2001-2010</td>
<td>Newly diagnosed high risk (24) (mostly &lt;5 y/o)</td>
<td>MB (21) PMET (3)</td>
<td>2</td>
<td>Thio → Thio</td>
<td>3 wks</td>
<td>3-y EFS: 79%</td>
<td>3-y OS: 82%</td>
</tr>
<tr>
<td>Korea</td>
<td>1999-2005</td>
<td>Relapse (11) Newly diagnosed high risk (14)</td>
<td>MB (18) PMET (7)</td>
<td>1-2</td>
<td>Mel + endoxan → Thio + carbo + VP16</td>
<td>12 wks</td>
<td>3-y EFS: (&gt;3 y/o and fresh) 83% (relapse) 29%</td>
<td>3-y OS: (&gt;3 y/o and fresh) 83% (relapse) 26%</td>
</tr>
<tr>
<td>COG99703</td>
<td>1998-2004</td>
<td>Fresh and &lt;3 y/o (92)</td>
<td>MB (38) PMET (17) ATRT (8) Others (31)</td>
<td>3</td>
<td>Thio + carbo → Thio + carbo → Thio + carbo</td>
<td>3 wks</td>
<td>5-y EFS: 44%</td>
<td>5-y OS: 64%</td>
</tr>
<tr>
<td>CGG</td>
<td>1995-2002</td>
<td>Relapse (32)</td>
<td>MB (18) PMET (1) Others (13)</td>
<td>2</td>
<td>Thio + BCNU → Thio + carbo</td>
<td>4-6 wks</td>
<td>3-y EFS: 25%</td>
<td>3-y OS: 38%</td>
</tr>
</tbody>
</table>

**Notes:**
- ATRT = atypical teratoid rhabdoid tumor; COG = Children Cancer Group; COG = Children Oncology Group; EFS = event-free survival; HSCT = hematopoietic stem cell transplant; MB = medulloblastoma; OS = overall survival; PFS = progression-free survival; PMET = primitive neuroectodermal tumor; VP16 = etoposide.
The lipophilic nature of thiotaepa and better BBB penetration led to the investigation of its use combined with stem cell rescue to increase dose intensity of chemotherapy for better tumor control in central nervous system tumors. In the literature, many investigators describe the use of thiotaepa-based conditioning regimens in the setting of single transplant, mostly melphalan-based chemotherapy, which has also been used as a conditioning regimen in other childhood solid cancers. While using tandem transplant as part of consolidation in those patients, most transplants relied on two or three successive courses of a thiotaepa-based regimen during tandem transplant at an interval of 3-8 weeks (Table 3). Investigators in Korea used a different approach by alternating a thiotaepa-based or melphalan-based conditioning regimen in double transplant at an interval of 12 weeks; however, no studies have yet established the optimal use of a conditioning regimen for patients with embryonal tumors in the setting of either single or tandem transplant.

Because thiotaepa is not readily available in our country, and because of its high price without insurance reimbursement in our healthcare system, we did not routinely administer a thiotaepa-based regimen during tandem transplant. Comparisons of possible benefits, risks, and costs of a thiotaepa-based versus a melphalan-based regimen were discussed with parents, which created the opportunity to observe the differences in outcome between patients receiving a thiotaepa-based regimen or those not receiving it. Our study, although a retrospective analysis with a small number of patients, identified a difference in outcome in pediatric patients with high-risk embryonal brain tumors receiving tandem thiotaepa-based regimens for one or more cycles in both PFS (Figure 1D) and OS (not shown) (p < 0.001 for PFS, p = 0.001 for OS). Two patients who chose to use non-thiotaepa-based conditioning regimens in double transplant eventually relapsed within 6 months, while four patients were disease free after tandem transplant using at least one cycle of thiotaepa-based regimen with a median PFS of 16.1 months (range, 7.2-51.8 months), for a total of nine patients.

The limitations of our study include its retrospective nature, small number of cases, and different disease entities, which might prevent us from formulating a universal recommendation. With enrollment of more cases of a uniform disease entity in a prospective setting, the differences in outcome between use of thiotaepa and lack thereof, and the effect of withholding RT among different embryonal brain tumors in patients receiving tandem transplant might be revealed. In conclusion, the data in our series shows that tandem transplant is feasible and safe for the treatment of high-risk embryonal brain tumors in young children, with a manageable toxicity profile. Combined with salvage RT, a favorable 2-year OS could be achieved in the majority of patients in the study. Patients receiving tandem transplant for one or more cycles of thiotaepa-based regimen might have better outcome than those not receiving this treatment.

REFERENCES


Multisection computed tomography: Results from a Chinese survey on radiation dose metrics

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Abstract

Background: As multisection spiral computed tomography (MSCT) have been extensively used, it is important to consider the amounts of doses the patients are exposed during a computed tomography (CT) examination. The aim of the current study was to summarize MSCT doses in Chinese patients to establish the diagnostic reference levels (DRLs).

Methods: Radiation dose metrics were retrospectively collected from 164,073 CT examinations via the Radimetrics Enterprise Platform. Radiation dose metrics (volume CT dose index [CTDvol], dose-length product [DLP], effective dose [ED], and organ dose) and size-specific dose estimate (SSDE) were calculated for adults and children based on anatomic area and scanner type.

Results: The median CTDvol and DLP values were highest in the head at 51.7 mGy (interquartile range [IQR], 33.2-51.7 mGy) and 906.5 mGy·cm (IQR, 582.4-1068.2 mGy·cm) and lowest in the chest at 7.9 mGy (IQR, 7.9-10.3 mGy) and 284.8 mGy·cm (IQR, 249.0-412.6 mGy·cm), respectively. The median SSDE values of chest and pelvis were 12.1 mGy (IQR, 10.8-14.1 mGy) and 36.3 mGy (IQR, 34.0-38.9 mGy), respectively. EDs for children were similar to adults except for an increased 1.5-, 0.77-, and 1.7-fold in the chest, neck, and pelvis, respectively (p < 0.001). Furthermore, radiation doses tended to increase with increasing slice number and decrease when exposure reduction techniques were used.

Conclusion: Our findings provide a basis for the evaluation of CT radiation doses and evidence for establishment of DRLs in China.

Keywords: Diagnostic reference levels; Dose-length product; Effective dose; Radiation dose metrics; Volume CT dose index

1. INTRODUCTION

The development of multisection (multislice) spiral computed tomography (MSCT) has led to a noticeable quantum leap in clinical performance of computed tomography (CT), enabling faster and accurate diagnosis of diseases. Nevertheless, the associated high radiation dose of CT is a major concern regarding an increased risk of carcinogenesis in the receivers, especially in pediatric patients. Radiation doses from CT are highly dependent on the number, operation condition, examination, and protocol. The most efficient CT types are often associated with a high risk of carcinogenesis due to high radiation efficiency. Although it is mandatory to ensure safety against ionizing radiation during the procedure, general dose limits cannot be utilized for CT examinations as the potential risks and benefits must be weighed on an individual basis.

2. METHODS

2.1. Study design and population

In this retrospective study, radiation doses of 169,802 MSCT examinations performed by eight CT scanners between July 2014 and March 2016 were included. The study was approved by the local institutional review board, and written informed consent was obtained from all patients. The radiation dose data were obtained from the Radimetrics Enterprise Platform, which monitors, tracks, and manages patient radiation exposures. The radiation doses were calculated using the dose-length product (DLP), effective dose (ED), and organ dose. The size-specific dose estimate (SSDE) was also calculated for adults and children based on anatomic area and scanner type.
2015 and January 2016 were analyzed to estimate the radiation dose metrics for adult and pediatric patients, which may assist the clinicians to set DRLs for Chinese patients. A total of 164,073 examinations from patients who underwent MSCT were included, after excluding CT examinations (5729 examinations) that lacked complete information on age, anatomical sites and clinical indications, clear indication, and clear definition of the scanned area; positron emission tomography/CT examinations; and MSCT examinations performed for research or interventional procedures. Examinations were grouped according to whether they were performed on teenagers and adults (aged >14 years) or children (aged ≤14 years).

The study was approved by the ethical committee of our hospital, and the requirement to obtain informed consent was waived because of the retrospective design of the study.

2.2. CT scanners
Eight CT (The General Electric Company, Waukesha, WI 53188, USA) facilities in the hospital from three manufacturers were included in this study: three from Siemens (Siemens Medical Solutions, Malvern, PA, USA), two from GE Healthcare, and three from Philips (Philips Medical Systems Nederland BV, a Philips Healthcare company, Best, The Netherlands) (Appendix 1). We divided these CT facilities into three groups based on the number of slices and whether dose-saving techniques were used or not: CT group A (CT 1, 2, 3, 7) were 64 sliced and above, CT group B (CT 4, 5) were 16 sliced, and group C (CT 6, 8) were 64 sliced using radiation exposure reduction algorithms including Adaptive Statistical Iterative Reconstruction (ASIR, for CT6) and iDose (for CT8), which improve the image quality and allow the use of lower tube currents (data not shown). A total of 95 experienced radiologists were involved in scanning and reading of CT images.

2.3. Data collection and CT protocol
Radiation dose data from MSCT examinations were collected and downloaded from Radimetrics Enterprise Platform (Bayer Healthcare, Whippany, NJ, USA) for analysis. Radimetrics collects dose metrics from the Digital Imaging and Communications in Medicine and Picture Archiving and Communications System (PACS) and derives the size-specific dose estimate (SSDE) by calculating patient diameter from the mid-scan length. Radimetrics uses the library of Cristy phantoms to calculate the ED by matching patients to a particular computational phantom based on the patient’s age, weight, or diameter. For different scanning protocols with various examination parameters, a set of Monte Carlo simulations are pruned for every phantom in the library to calculate organ doses, which are then used to derive the ED, according to the published ICRP 103 tissue-weighting factors. The radiation dose metrics such as volume CT dose index (CTDIvol), DLP, SSDE, ED, and organ doses between multiple groups were analyzed and compared between two groups using Kruskal-Wallis test and Wilcoxon rank-sum test using SAS version 9.4 (SAS Institute, Cary, NC, USA). A p value of ≤0.05 was considered to be statistically significant.

3. RESULTS

3.1. Patients demographics
A total of 164,073 patients were examined for radiation dose metrics (adults: n = 153,149; adult men: n = 86,791). The median age of all patients was 52.18 (37.85-62.73) years.

3.2. CT image distribution and radiation doses in all patients
Overall, the most common areas imaged were the chest (38.60%), head (31.04%), abdomen (23.43%), spine (4.24%), neck (0.78%), and pelvis (0.50%). Apart from these, only 1.42% of the examinations were of other anatomic areas (Figure 1A). The median radiation doses and IQRs are detailed in Table 1. The median CTDIvol values were highest in the head at 51.7 mGy (IQR, 33.2-51.7 mGy) and lowest in the chest at 7.9 mGy (IQR, 7.9-10.3 mGy). Similarly, the median DLPs were 906.5 mGy·cm (IQR, 582.4-1068.2 mGy·cm) in the head and 284.8 mGy·cm (IQR, 249.0-412.6 mGy·cm) in the chest. The median SSDE values were lowest in the chest at 12.1 mGy (IQR, 10.8-14.1 mGy) and highest in the pelvis at 36.3 mGy (IQR, 34.0-38.9 mGy). The median EDs were highest in the abdomen at 16.7 mSv (IQR, 12.7-22.4 mSv) and lowest in the head at 2.3 mSv (IQR, 1.5-2.7 mSv).

3.3. CT image distribution and radiation doses in adults and children
There were slight differences between adults and children in the most commonly imaged areas (Figure 2). In adults, the most common areas were the chest (37.1%), head (26.8%), abdomen (23.0%), spine (4.2%), neck (0.7%), and pelvis (0.5%). In children, there were significant differences in radiation dose metrics between adults and children in all the areas, except CTDIvol and DLP of the pelvis and EDs of the abdomen, spine, and neck (Table 2). The EDs were significantly high in children compared with adults with 1.5- and 1.7-fold in the head and pelvis, respectively (p < 0.001 for all).

3.4. CT image distribution and radiation doses in different groups
Different CT group were dispersed based on patients’ triage to examine different anatomic areas (Figure 1C). A total of

SSDE dose is used for body CT to account for differences in patient size, especially when comparing dose levels from different organizations that may have significant differences in patient demographics (affecting size or weight).

ED is calculated based on the organs exposed by the applied radiation multiplied by tissue-weighting factors. In Radimetrics, the organ doses are first calculated using Monte Carlo probabilistic simulations that account for scattered radiation using a library that includes standardized male and female anthropomorphic mathematical phantoms, then the ED is estimated according to the published ICRP103 tissue-weighting factors. Patient sex, age, and time of the examination, scan region (head, chest, abdomen, spine, neck, pelvis, and other anatomic areas), study description, protocol name, scanner manufacturer, and model were extracted from Radimetrics and PACS.

2.5. Statistical analysis
The study findings are presented as median, upper and lower quartile (interquartile range, IQR). Radiation dose metrics, CTDIvol, DLP, SSDE, ED, and organ doses between multiple groups were analyzed and compared between two groups using Kruskal-Wallis test and Wilcoxon rank-sum test using SAS version 9.4 (SAS Institute, Cary, NC, USA). A p value of <0.05 was considered to be statistically significant.

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103,149 CT examinations were collected from group A and 14,798 examinations were collected from group C. For group B, these two facilities were mostly used for routine head (18,149, 11.1%) and chest (27,871, 17.0%) examinations, and only 9 (<0.1%) and 91 (0.1%) patients had their abdomen or spine scanned. There were 1574 (1.0%), 6 (<0.1%), and 289 (0.2%) examinees in groups A, B, and C for other anatomic areas, respectively.

The median radiation doses and IQRs in each group are reported in Table 3. Among the three teams, CTDIvol and DLP of the chest ([9.8 versus 7.9 versus 8.8 mGy] and [387.8 versus 262.1 versus 334.5 mGy·cm], respectively) and the spine ([19.9 versus 21.3 versus 16.6 mGy] and [564.6 versus 1023.6 versus 417.6 mGy·cm], respectively) were significantly different ($p<0.0001$ for all). Abdominal and pelvic CTDIvol (18.2 versus 13.8 mGy) and (26.7 versus 15.4 mGy) and DLP (894.4 versus 709.2 mGy·cm) and (502.1 versus 338.3 mGy·cm) differed significantly only between groups A and C ($p<0.0001$, for both regions). CTDIvol of the head in group B were significantly lower only compared with team A (33.2 versus 51.7 mGy; $p<0.0001$).

Overall, it was apparent that radiation dose tended to increase as slice number increased from group B to A, and doses tended to reduce with the use of exposure reduction techniques in group C.

3.5. Radiation doses in different organs

The median organ doses were listed in Figure 2. During the ED calculation, the Monte Carlo simulations were used by Radimetrics for different scanning protocols with various examination parameters. Using the Monte Carlo simulations, the median highest dose received by the head was 24.8 mGy in the eye lenses and 18 mGy in the brain. The lowest doses were received by the pelvis and the ovaries at 4.2 mGy, bladder at 3.2 mGy, and uterus at 2.8 mGy.

### Table 1: Radiation dose metrics of all patients

<table>
<thead>
<tr>
<th>Anatomical region</th>
<th>Median CTDIvol, mGy* (IQR)</th>
<th>Median DLP, mGy·cm* (IQR)</th>
<th>Median SSDE, mGy* (IQR)</th>
<th>Median effective dose, mSv* (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td>51.7 (33.2-51.7)</td>
<td>906.5 (582.4-1068.2)</td>
<td>36.2 (19.1-44.0)</td>
<td>0.2 (0.1-0.3)</td>
</tr>
<tr>
<td>Chest</td>
<td>07.0 (7.9-10.3)</td>
<td>294.2 (249.0-412.6)</td>
<td>12.1 (10.8-14.1)</td>
<td>0.0 (0.0-0.0)</td>
</tr>
<tr>
<td>Abdomen</td>
<td>18.2 (14.6-18.5)</td>
<td>886.9 (647.8-1259.9)</td>
<td>24.5 (20.4-28.0)</td>
<td>0.0 (0.0-0.0)</td>
</tr>
<tr>
<td>Spine</td>
<td>19.9 (18.1-19.9)</td>
<td>552.6 (498.9-634.4)</td>
<td>...</td>
<td>16.7 (12.7-22.4)</td>
</tr>
<tr>
<td>Neck</td>
<td>12.6 (10.9-14.6)</td>
<td>356.0 (277.2-534.0)</td>
<td>...</td>
<td>13.2 (10.5-15.4)</td>
</tr>
<tr>
<td>Pelvis</td>
<td>26.7 (26.7-26.8)</td>
<td>498.2 (387.1-670.8)</td>
<td>36.3 (34.0-38.9)</td>
<td>0.0 (0.0-0.0)</td>
</tr>
</tbody>
</table>

* $p<0.0001$ among different anatomic areas compared with chest.

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4. DISCUSSION

The disproportionate increase in radiation-induced cancer risk compared with the benefits of CT has been a challenge for its use, especially in children worldwide. A number of CT dose surveys have been published worldwide based on this. However, Asian surveys have focused on only protocols or phantoms. To the best of our knowledge, this is the first large-sample patient survey that could be the basis for developing radiation dose standards in China. As there are no specific national reference levels in China, we compared the DRLs with DRLs from European guidelines. Our results showed similar or lower results on CTDIvol and a higher DLP, the latter probably occurring due to the longer scan length, as DLP is dependent on scan length, whereas CTDI is almost independent. Also, we tried to compare the dose metric parameters of this study with that of the study by Zhou et al., who surveyed adult patients from the Jiangsu province of China for CT radiation doses for more ethnic generalizability of results. Compared with the Zhou et al. findings, our study had high CTDIvol values for head (31.7 mGy versus 44.54 mGy) and low for chest (7.9 mGy versus 17.31 mGy) anatomical segments. Similarly, the DLP of head (906.5 mGy·cm versus 493.16 mGy·cm) was high and low for chest (284.8 mGy·cm versus 408.96 mGy·cm) in our study compared with the findings of Zhou et al. Compared with CTDIvol and DLP, ED is widely used, as it is the only measure of dose that can be easily compared with radiation dose measurements from other imaging tests and environmental exposures. When the EDs were compared with Zhou et al. findings, and the US and UK DRLs, the EDs observed in this study were slightly lower than that of doses from the UK and US population and were higher than the EDs of those from the study of Zhou et al. This discrepancy may be due to the smaller population surveyed in the study by Zhou et al. (n = 243) from a
single province in China. Moreover, individual body or organ surface area may have played a crucial role on the outcome. A study by Li et al. reported that the organ dose and ED decreased with increased organ (chest) diameter. Given that the Zhou et al. study involved only adult patients who tend to have larger organ diameter than pediatric patients. It is sensible that the high EDs and CTDIs obtained in this study may be due to the enrollment of both adult and pediatric patients who receive increased.

Table 2
Radiation dose metrics in children and adult patients

<table>
<thead>
<tr>
<th>Anatomical region</th>
<th>Age Group</th>
<th>Median CTDIvol, mGy² (IQR)</th>
<th>p</th>
<th>Median DLP, mGy·cm (IQR)</th>
<th>p</th>
<th>Median SSDE, mGy (IQR)</th>
<th>p</th>
<th>Median effective dose, mSv (IQR)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td>Children</td>
<td>51.5 (33.2-51.7)</td>
<td>&lt;0.001</td>
<td>989.0 (582.4-1047.3)</td>
<td>0.0001</td>
<td>...</td>
<td>...</td>
<td>03.4 (2.2-4.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adults</td>
<td>51.7 (33.2-51.7)</td>
<td>...</td>
<td>906.5 (582.4-1071.9)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>02.3 (1.4-2.6)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Chest</td>
<td>Children</td>
<td>04.3 (3.1-7.9)</td>
<td>&lt;0.0001</td>
<td>110.1 (65.2-206.3)</td>
<td>&lt;0.0001</td>
<td>08.3 (6.5-14.2)</td>
<td>&lt;0.0001</td>
<td>05.5 (4.0-8.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adults</td>
<td>07.9 (7.3-10.4)</td>
<td>...</td>
<td>286.8 (252.7-420.0)</td>
<td>...</td>
<td>12.1 (10.9-14.1)</td>
<td>...</td>
<td>07.1 (6.1-8.6)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Abdomen</td>
<td>Children</td>
<td>18.2 (6.9-18.2)</td>
<td>&lt;0.0001</td>
<td>526.1 (283.0-757.4)</td>
<td>&lt;0.0001</td>
<td>28.6 (13.4-36.0)</td>
<td>0.0006</td>
<td>18.6 (13.2-27.1)</td>
<td>0.4877</td>
</tr>
<tr>
<td>Adults</td>
<td>18.2 (14.7-18.6)</td>
<td>...</td>
<td>893.3 (658.0-1272.0)</td>
<td>...</td>
<td>24.5 (20.5-27.9)</td>
<td>...</td>
<td>16.7 (12.6-22.1)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Spine</td>
<td>Children</td>
<td>19.9 (6.6-19.9)</td>
<td>&lt;0.0001</td>
<td>362.5 (173.7-534.5)</td>
<td>&lt;0.0001</td>
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<td>...</td>
<td>15.1 (5.6-19.4)</td>
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<tr>
<td>Adults</td>
<td>19.9 (18.3-19.9)</td>
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<td>890.0 (502.9-636.0)</td>
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<td>...</td>
<td>...</td>
<td>13.2 (10.6-15.4)</td>
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<td></td>
</tr>
<tr>
<td>Neck</td>
<td>Children</td>
<td>09.5 (7.9-11.2)</td>
<td>&lt;0.0001</td>
<td>906.5 (126.8-284.9)</td>
<td>&lt;0.0001</td>
<td>...</td>
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<td>03.7 (2.6-5.8)</td>
<td>0.3203</td>
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<tr>
<td>Adults</td>
<td>12.9 (11.1-15.0)</td>
<td>...</td>
<td>110.1 (287.1-550.0)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>03.6 (2.8-6.0)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Pelvis</td>
<td>Children</td>
<td>26.7 (26.4-26.8)</td>
<td>0.1555</td>
<td>286.8 (285.5-584.8)</td>
<td>0.2616</td>
<td>46.1 (41.3-51.0)</td>
<td>&lt;0.0001</td>
<td>13.8 (10.4-16.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adults</td>
<td>26.7 (26.7-26.8)</td>
<td>...</td>
<td>526.1 (387.6-674.9)</td>
<td>...</td>
<td>36.2 (33.8-38.8)</td>
<td>...</td>
<td>07.9 (5.8-10.4)</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.0001 among different anatomic areas compared with chest.

Table 3
Radiation dose metrics of patients in different CT scanner group

<table>
<thead>
<tr>
<th>Scanner teams</th>
<th>Anatomical region</th>
<th>Median CTDIvol, mGy²</th>
<th>IQR</th>
<th>Median DLP, mGy·cm</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Team A</td>
<td>Head</td>
<td>51.7</td>
<td>51.5-51.7</td>
<td>1046.4</td>
<td>995.0-1103.7</td>
</tr>
<tr>
<td></td>
<td>Chest</td>
<td>9.9</td>
<td>7.4-13.5</td>
<td>387.8</td>
<td>284.0-556.1</td>
</tr>
<tr>
<td></td>
<td>Abdomen</td>
<td>18.2</td>
<td>15.1-18.5</td>
<td>894.4</td>
<td>663.6-1281.0</td>
</tr>
<tr>
<td></td>
<td>Spine</td>
<td>19.9</td>
<td>19.6-19.9</td>
<td>564.6</td>
<td>528.1-643.0</td>
</tr>
<tr>
<td></td>
<td>Neck</td>
<td>12.6</td>
<td>10.9-14.5</td>
<td>355</td>
<td>277.3-532.3</td>
</tr>
<tr>
<td></td>
<td>Pelvis</td>
<td>26.7</td>
<td>26.7-26.8</td>
<td>502.1</td>
<td>391.0-674.9</td>
</tr>
<tr>
<td>Team B</td>
<td>Head</td>
<td>33.2</td>
<td>32.2-33.2</td>
<td>582.4</td>
<td>516.0-582.4</td>
</tr>
<tr>
<td></td>
<td>Chest</td>
<td>7.9</td>
<td>7.9-7.9</td>
<td>262.1</td>
<td>240.1-280.9</td>
</tr>
<tr>
<td></td>
<td>Abdomen</td>
<td>21.4</td>
<td>21.4-21.4</td>
<td>878.2</td>
<td>439.7-985.2</td>
</tr>
<tr>
<td></td>
<td>Spine</td>
<td>21.3</td>
<td>21.3-21.3</td>
<td>1023.6</td>
<td>690.7-1407.1</td>
</tr>
<tr>
<td></td>
<td>Neck</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Pelvis</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Team C</td>
<td>Head</td>
<td>51.9</td>
<td>51.5-51.9</td>
<td>726.7</td>
<td>726.7-830.5</td>
</tr>
<tr>
<td></td>
<td>Chest</td>
<td>8.8</td>
<td>6.3-12.4</td>
<td>334.5</td>
<td>243.2-468.2</td>
</tr>
<tr>
<td></td>
<td>Abdomen</td>
<td>13.8</td>
<td>10.8-18.2</td>
<td>709.2</td>
<td>487.6-1032.4</td>
</tr>
<tr>
<td></td>
<td>Spine</td>
<td>16.6</td>
<td>10.4-19.1</td>
<td>417.6</td>
<td>311.2-506.5</td>
</tr>
<tr>
<td></td>
<td>Neck</td>
<td>14.7</td>
<td>8.5-15.4</td>
<td>468.4</td>
<td>271.2-761.1</td>
</tr>
<tr>
<td></td>
<td>Pelvis</td>
<td>15.4</td>
<td>10.9-21.8</td>
<td>338.3</td>
<td>280.6-570.6</td>
</tr>
</tbody>
</table>

*p < 0.0001 among three groups in the same anatomic area.

*p < 0.0001 between groups A and C in the same anatomic area.
radiation doses. Moreover, the findings of this survey show that EDs for children were similar to those for adults in the abdomen, neck, and spine, but increased approximately 1.5-fold in the head and 1.7-fold in the pelvis. This is in line with the study by Thomas and Wang, who reported higher ED estimates for younger age groups than older age groups for head, abdomen, and pelvis MSCT scans. Further, the relationship between ED and stochastic risk is assumed to be linear34 and the risk of carcinogenesis is estimated to increase proportionally with organ dose.35,36 Furthermore, we should also notice that the number of abdominal scans was small, especially in children (732 cases [0.45%]), as many children would have opted for other imaging techniques such as magnetic resonance (MR) and ultrasound. In addition, MR reports were also preferred for complicated cases in adult patients who underwent CT for cancer staging before surgery. In contrast, we report similar or lower doses to previous values reported for the head, chest, neck, and pelvis.37,38 Due to their lower body weight and sizes, children often receive higher ED than adults when adult-size imaging techniques and protocols are used.39 Usage of age- and child-specific protocols,39 optimizing scan parameters based on patient anatomy, and reducing the number of multiphase scans can go a long way in reducing ED in children.39 Radiation doses showed an obvious tendency to increase with slice number and decrease with the use of exposure reduction techniques such as ASIR and iDose. Further, there was an increasing trend toward radiation dose with increasing number of slices, especially in the head and chest, which is in contrast with a study in which dose reduction was achieved for all types of CT examinations with the 236-slice scanner.40 However, the results were similar to the doses associated with 4-, 8-, 16-, and 64-slice CT scanners.41 Hence, small slice CT scanners and large-sliced scanners with exposure reduction techniques such as ASIR and iDose may be used efficiently to scan anatomical areas with low radiation doses. It should be noted that although CTDIvol and DLP were higher in the head compared with the chest in our study, the latter was higher in terms of ED. CTDIvol measures the radiation output of a CT scanner, which is useful to compare devices. However, CTDIvol depends on tube current, which changes as the type of scan being performed. Since the penetrating power required to visualize the brain is higher compared with the chest due to its anatomy, the tube current used is higher, causing the CTDIvol of head scans to rise. On the other hand, ED is a measure of the dose received by the patient, with tissue-weighting factors coming into play. This factor is smaller for the head compared with the chest (0.0021 versus 0.014 mSv·mGy·cm),42 causing the ED received during head scans to be much lower as compared with chest scans. Hence, the patients who had chest scans received more radiation than patients who had head scans. Furthermore, since the incidence of cancer has been reported to be larger after chest scans,43 it is possible that such patients in our study could also be at risk.

There are several limitations to our study. It was a retrospective, single-center study, similar to many other dose surveys, resulting in an inherent bias in patient selection. The number of examinations included in the evaluation was small compared with the total examinations in our hospital. The time of observation was only 6 months, given the recent introduction of Radimetrics in China. Because of the large number of patients (about 1000 examinations per day) undergoing CT, we had to distribute them into different CT groups: physical examinations and spine CT group and none in the neck and pelvis group. Furthermore, although pediatric-specific CT protocols were used in this study, they were not well optimized as also seen in previous studies.12,44 However, adult CT protocols were not used in pediatric patients. Lastly, the CT equipment used in our analysis were from different manufacturers and had different use situations; for example, CT4 was used for lung scanning because of the larger number of patients, whereas CT5 was used mostly for head and few lungs scans. This led to difficulties in analyzing the scans from the two scanners. In the future studies, we also plan to add more equipment for analysis in case of increase in the patient sample size. Nevertheless, this first survey in China to estimate the radiation doses may be of significant importance for future studies and also clinicians to set DRLs for patients.

In conclusion, the findings of this study reveal the radiation doses in China for a large number of observations using automated data collection. These data provide a basis for evaluation of CT radiation doses in China and allow institutions to understand doses by anatomical area to develop DRLs and allow for cross-country comparisons.
Health-related quality of life in children and adolescent with different types of scoliosis: A cross-sectional study

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Abstract

Background: The health-related quality of life (HRQoL) was affected in children and adolescents with scoliosis. However, there was lack of study to compare the HRQoL among patients with different types of scoliosis. We aimed to investigate whether the HRQoL differs among patients with idiopathic, congenital, neuromuscular, and syndromic scoliosis.

Methods: Children and adolescents with scoliosis were recruited from a single tertiary hospital. The HRQoL, as assessed by the child health questionnaire 50-item parent form, was compared with a reference health sample group using the effect size (ES). Intergroup differences related to scoliosis subtype and severity were explored.

Results: A total of 67 participants with scoliosis (24 idiopathic, 15 congenital, 15 neuromuscular, and 13 syndromic) were analyzed. The HRQoL in patients with neuromuscular scoliosis was affected the most, in both physical (ES range: 0.97–2.4) and psychosocial domains (ES range: 0.92–2.58). To a lesser extent, the physical (ES range: 0.99–1.13) and psychosocial (ES range: 0.8–1.18) domains were also affected in patients with syndromic scoliosis. The domains of family activities (ES = 1.1), role/social–emotional/behavioral (ES = 0.99), general health perception (ES = 0.94), and self-esteem (ES = 0.87) were affected in patients with idiopathic scoliosis. In contrast, only the general health perception domain (ES = 1.27) was affected in patients with congenital scoliosis. Scoliosis severity correlated with scores in the physical domains and some psychosocial domains, while treatment type correlated with scores in the physical domains only. Scoliosis subtype and severity both affected the physical and psychosocial domains, with a stronger impact for subtype.

Conclusion: Differences in the HRQoL exist among scoliosis subtypes, with neuromuscular scoliosis being most affected. Although the scoliosis subtype and severity both affect the HRQoL, the subtype is more influential than severity.

Keywords: Congenital scoliosis; Health-related quality of life; Idiopathic scoliosis; Neuromuscular scoliosis; Syndromic scoliosis

1. INTRODUCTION

Scoliosis is a three-dimensional deformity of the spine, clinically defined as a curvature of the spine greater than 10 degrees in the coronal plane. It is often accompanied by a rotation of the spine in the axial plane. Scoliosis can be classified into congenital, idiopathic, neuromuscular, syndromic, and functional types.1

Congenital scoliosis (CS) is a malformation resulting from a prenatal disruption of vertebral formation or segmentation, leading to imbalanced longitudinal growth and rotation of the vertebrae. The symptoms of CS are usually not observed at birth; however, impaired ambulation may be observed following vertebral rotation during development.2

Idiopathic scoliosis (IS) is the most common type of scoliosis, accounting for more than 80% of scoliosis cases.3 The diagnosis requires exclusion of other anatomic anomalies. IS may be divided into three subtypes according to the age of onset: infantile, juvenile, and adolescent. The subgroups differ in their progression and treatment. The infantile type occurs within the first 3 years of life and often resolves spontaneously. The juvenile type occurs between 3 and 9 years of age, and most of these children require intervention.4 Adolescent IS (AIS) has an onset after 10 years of age and is the most common type of IS; in most of these patients, the clinical course is not serious, with intervention required in only 10%.5

Neuromuscular scoliosis (NMS) is of multiple etiologies and the incidence is variable.6 The severity of the spinal curve deformity is related to the degree of neuromuscular involvement. Cerebral palsy (CP), muscular dystrophy, spinal cord injury, and spinal dysraphism are common etiologies. NMS usually develops early and may progress quickly in certain conditions, such as tethered spinal cord syndrome, hydrocephalus, or intraspinal tumor.

Syndromic scoliosis (SS) co-occurs with many genetic and nongenetic syndromes, including VACTERL association, Marfan syndrome, Ehlers-Danlos syndrome, neurofibromatosis, Rett syndrome, and Down syndrome. The cause, symptoms, and progression of SS vary depending on the disease context.

While the etiology, onset, prognosis, and treatments vary among these classifications, the possible outcomes of scoliosis are similar: respiratory compromise, seating compromise, pain, gait impairment, difficulty with activities of daily living,
and psychological distress. The health-related quality of life (HRQoL) may thus be jeopardized.

Although the HRQoL is an important issue in current clinical practice, its quantification has not been standardized. Several questionnaires, such as the SRS-22, EQ-D5, SF-36, and Muscular Dystrophy Spine Questionnaire, have been used in evaluating the HRQoL of patients with scoliosis. Earlier studies have demonstrated that AIS can lead to increased physical, psychological, and social problems in patients. Factors such as pain, restricted physical activities, poor body image, maladjustment in school, and poor peer relationships may consequently contribute to a decreased quality of life in adolescents with scoliosis.

The child health questionnaire (CHQ) is a HRQoL measurement tool comprising physical and psychosocial domains. The CHQ is based on the parents’ perceptions, which represent a subjective vision of the patient’s HRQoL. The CHQ has been translated into several languages, and its validity and reliability have been evaluated in the United Kingdom, Germany, Francophone Canada, Australia, Norway, Italy, and the Netherlands. The CHQ may be applied in the general population and in chronically ill children. Nixon et al. confirmed the validity of the CHQ among survivors of childhood cancer. Westendorp et al. surveyed the responsiveness of the CHQ in adolescents with chronic pain or fatigue. Other studied populations include children with upper- and lower-transplantation, and cystic periventricular leukomalacia. In these studies, clinical practitioners found the CHQ to be a useful tool for evaluating both the physical and psychosocial aspects of the quality of life.

A frequently applied version of the CHQ is the child health questionnaire 50-item parent form (CHQ-PF50), which is a parent-completed questionnaire designated for children 5–18 years of age. To our knowledge, the CHQ-PF50 has not yet been used to compare the HRQoL among children and adolescents with different types of scoliosis. Therefore, the primary aim of the present study was to evaluate whether the HRQoL, as assessed by the CHQ-PF50, differs among four types of scoliosis. Furthermore, we assessed the relationships between CHQ-PF50 scores and potential correlated factors and examined the interaction between scoliosis type and severity in the affected CHQ-PF50 domains.

2. METHODS

2.1. Study population

The study was conducted in a tertiary medical center in Taiwan, in design of cross-sectional study. Patients with a confirmed diagnosis of IS, CS, NMS, or SS, 5–18 years of age, were enrolled during their appointments at the scoliosis clinic, when hospitalized for surgery, or when under surveillance for scoliosis. The patients provided informed consent. The recruitment period was between May 2014 and December 2016. The data of control group were derived from a earlier Taiwanese study. A total 129 healthy children (95 boys, 103 girls), 6–15 of age, by means of written requests and mail-back questionnaires in 2006, were chosen as the control group. The study protocol was approved by the local Institutional Review Board.

2.2. Measures

The severity of the scoliosis was defined using Cobb’s angle (mild: 10º–24º; moderate: 25º–40º; and severe: >40º). The HRQoL was measured using the traditional Chinese (Taiwan) version of the CHQ-PF50 (HealthActCHQ Inc., Boston, MA). The CHQ-PF50 consists of 50 questions to assess the health status of children, according to 12 physical and psychosocial domains. Each question was answered according to a five-point scale, and was intended to assess the previous 4 weeks’ performance, except when there was a change in health, in which case it covered the most recent year of the child’s life. Each domain was scored from 0 to 100, with a higher score indicating better health. The domains were further transformed into two main scores: the physical summary score (PhS) and psychosocial summary score (PsS), with a mean of 50 and a standard deviation of 10. Physical health comprises four components: physical functioning, role/social–physical, general health perception, and bodily pain. Psychosocial health comprises four components: role/social–emotional/behavioral, self-esteem, mental health, and behavior. Two scales (parental impact–time and parental impact–emotion) contribute to both dimensions but have a stronger correlation with psychosocial health. The final two subscales focus on family activities and family cohesion.

2.3. Procedure

Each patient underwent an image survey to confirm the scoliosis diagnosis; Cobb’s angle was measured by the same specialist for all patients. The types of treatment were documented. The parents then completed the CHQ-PF50.

2.4. Statistical analyses

Demographic characteristics were summarized using descriptive statistics. Continuous variables are presented as means and standard deviation. Categorical variables are presented as numbers and percentage. Effect sizes (ESs) were compared with those in an earlier study involving healthy Taiwanese children. An ES > 0.8 was considered to be large. Spearman’s correlation analyses were performed to identify associations between CHQ-PF50 scores and age, severity, and treatment type. A multivariable analysis was performed to evaluate the interaction between scoliosis type and severity and to clarify the possible confounding effects. The multivariable analysis was focused on domains with a significant intergroup difference as assessed using the Kruskal-Wallis test. Statistical analyses were performed using SPSS (Version 23, IBM Corporation, Armonk, NY) and a p < 0.05 was considered statistically significant for a two-tailed test.

3. RESULTS

Sixty-seven children and adolescents (45 girls and 22 boys) with scoliosis were enrolled. Twenty-four patients had IS, 15 patients had CS, 15 patients had NMS, and 13 patients had SS. The NMS group included patients with hydromyelia (n = 1), spinal tumor (n = 1), spinal dysraphism (n = 4), peripheral neuropathy (n = 1), neurofibromatosis (n = 1), CP (n = 4), spinal muscular atrophy (n = 1), Duchenne muscular dystrophy (n = 1), and basal ganglia germinoma (n = 1). The SS group included patients with Rett syndrome (n = 1), Marfan syndrome (n = 3), VACTERL association (n = 3), Apert syndrome (n = 1), Holt-Oram syndrome (n = 1), Angelman syndrome (n = 1), achondroplasia (n = 1), cerebro-costo-mandibular syndrome (n = 1), and cri-du-chat syndrome (n = 1).

There were no significant differences among the scoliosis subtypes for mean age, Cobb’s angle, sex distribution, curve severity, or treatment used (Table 1).

Table 2 shows the CHQ-PF50 scores of the study sample in comparison to those in the reference sample of healthy children. Patients with scoliosis had significantly lower scores in physical functioning, role/social–physical, role/social–emotional/behavioral, self-esteem, general health perception, and family activities, and lower PhS scores compared with those in healthy controls. In the IS group, only health perception scores were lower compared with those in healthy controls. The NMS and SS groups had lower scores compared with those in healthy controls in both physical and psychosocial multiple domains, while the IS group had lower scores in the psychosocial domains and general health perception of physical domain.

No domains or summary scores were correlated with age. Scores in the physical functioning (p < 0.001), role/social–physical (p = 0.002), bodily pain (p < 0.001), role/social–emotional/behavioral (p = 0.006), and family activities (p = 0.026) categories, as well as the PhS (p < 0.001), were significantly correlated...
Table 1
Demographic characteristics

<table>
<thead>
<tr>
<th></th>
<th>IS (N = 24)</th>
<th>CS (N = 15)</th>
<th>NMS (N = 15)</th>
<th>SS (N = 13)</th>
<th>Control (N = 129)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean ± SD)</td>
<td>12.8 ± 3.6</td>
<td>9.8 ± 4.6</td>
<td>11.7 ± 3.5</td>
<td>12.8 ± 3.7</td>
<td>10.1 ± 2.3</td>
</tr>
<tr>
<td>Cobb’s angle (degree, mean ± SD)</td>
<td>37.3 ± 18.3</td>
<td>43.7 ± 17.0</td>
<td>55.9 ± 32.6</td>
<td>46.8 ± 25.2</td>
<td>NA</td>
</tr>
<tr>
<td>Number (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5 (20.8)</td>
<td>5 (33.3)</td>
<td>6 (40.0)</td>
<td>6 (46.2)</td>
<td>95 (73.6)</td>
</tr>
<tr>
<td>Female</td>
<td>19 (79.2)</td>
<td>10 (66.7)</td>
<td>9 (60.0)</td>
<td>7 (53.8)</td>
<td>34 (26.4)</td>
</tr>
<tr>
<td>Severity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>5 (20.8)</td>
<td>3 (20.0)</td>
<td>2 (13.3)</td>
<td>4 (30.9)</td>
<td>NA</td>
</tr>
<tr>
<td>Moderate</td>
<td>8 (33.3)</td>
<td>3 (20.0)</td>
<td>4 (26.7)</td>
<td>1 (7.7)</td>
<td>NA</td>
</tr>
<tr>
<td>Severe</td>
<td>11 (45.9)</td>
<td>9 (60.0)</td>
<td>9 (60.0)</td>
<td>8 (61.5)</td>
<td>NA</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observation</td>
<td>11 (45.8)</td>
<td>5 (33.3)</td>
<td>5 (33.3)</td>
<td>7 (53.8)</td>
<td>NA</td>
</tr>
<tr>
<td>Brace</td>
<td>7 (29.2)</td>
<td>2 (13.3)</td>
<td>5 (33.3)</td>
<td>1 (7.7)</td>
<td>NA</td>
</tr>
<tr>
<td>Surgery</td>
<td>6 (25.0)</td>
<td>6 (53.3)</td>
<td>5 (33.3)</td>
<td>5 (38.5)</td>
<td>NA</td>
</tr>
</tbody>
</table>

CS = congenital scoliosis; IS = idiopathic scoliosis; NMS = neuromuscular scoliosis; SS = syndromic scoliosis; SD = standard deviation.

Table 2
Mean value and effect size of CHQ-PF50 domain and summary scores compared to that in a reference sample of healthy Taiwanese children

<table>
<thead>
<tr>
<th></th>
<th>IS (N = 24)</th>
<th>CS (N = 15)</th>
<th>NMS (N = 15)</th>
<th>SS (N = 13)</th>
<th>Total</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td><strong>SD</strong></td>
<td><strong>Mean</strong></td>
<td><strong>SD</strong></td>
<td><strong>Mean</strong></td>
<td><strong>SD</strong></td>
<td><strong>Mean</strong></td>
</tr>
<tr>
<td>Physical construct</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical functioning</td>
<td>69.35</td>
<td>17.56</td>
<td>90.4</td>
<td>26.10</td>
<td>0.32</td>
<td>33.33</td>
</tr>
<tr>
<td>Role/social-physical</td>
<td>63.80</td>
<td>22.93</td>
<td>84.4</td>
<td>26.50</td>
<td>0.49</td>
<td>38.89</td>
</tr>
<tr>
<td>Bodily pain</td>
<td>86.11</td>
<td>23.91</td>
<td>80.0</td>
<td>16.04</td>
<td>0.36</td>
<td>64.00</td>
</tr>
<tr>
<td>General health perception</td>
<td>60.00</td>
<td>15.17</td>
<td>94.5</td>
<td>16.81</td>
<td>1.27</td>
<td>42.83</td>
</tr>
<tr>
<td><strong>Psychosocial construct</strong></td>
<td><strong>Behavior</strong></td>
<td><strong>Role/social–emotional/behavioral</strong></td>
<td><strong>Mental health</strong></td>
<td><strong>Self-esteem</strong></td>
<td><strong>Parent impact–emotion</strong></td>
<td><strong>Parent impact–time</strong></td>
</tr>
<tr>
<td>Behavior</td>
<td>77.08</td>
<td>21.96</td>
<td>-0.48</td>
<td>74.8</td>
<td>13.07</td>
<td>0.48</td>
</tr>
<tr>
<td>Role/social–emotional/behavioral</td>
<td>68.96</td>
<td>15.09</td>
<td>0.99</td>
<td>86.7</td>
<td>26.29</td>
<td>-0.03</td>
</tr>
<tr>
<td>Mental health</td>
<td>73.33</td>
<td>16.53</td>
<td>0.52</td>
<td>86.0</td>
<td>11.53</td>
<td>-0.43</td>
</tr>
<tr>
<td>Self-esteem</td>
<td>70.31</td>
<td>21.43</td>
<td>0.87</td>
<td>71.7</td>
<td>23.00</td>
<td>0.75</td>
</tr>
<tr>
<td>Parent impact–emotion</td>
<td>61.11</td>
<td>26.43</td>
<td>0.47</td>
<td>62.7</td>
<td>27.25</td>
<td>0.38</td>
</tr>
<tr>
<td>Parent impact–time</td>
<td>62.50</td>
<td>28.72</td>
<td>0.46</td>
<td>79.3</td>
<td>22.95</td>
<td>-0.23</td>
</tr>
<tr>
<td>Family activities</td>
<td>68.58</td>
<td>24.05</td>
<td>1.10</td>
<td>83.1</td>
<td>17.57</td>
<td>0.41</td>
</tr>
<tr>
<td>Family cohesion</td>
<td>66.67</td>
<td>31.44</td>
<td>0.20</td>
<td>67.0</td>
<td>20.34</td>
<td>0.24</td>
</tr>
<tr>
<td>Physical summary score</td>
<td>47.43</td>
<td>11.43</td>
<td>0.49</td>
<td>46.1</td>
<td>12.35</td>
<td>0.60</td>
</tr>
<tr>
<td>Psychosocial summary score</td>
<td>44.23</td>
<td>10.75</td>
<td>0.61</td>
<td>48.7</td>
<td>11.20</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Mean value and effect size of CHQ-PF50 domain and summary scores compared to that in a reference sample of healthy Taiwanese children.

CS = congenital scoliosis; ES = effective size; IS = idiopathic scoliosis; NA = not applicable; NMS = neuromuscular scoliosis; SS = syndromic scoliosis; SD = standard deviation.

Table 3
Significant correlation between certain domains with severity and treatment

<table>
<thead>
<tr>
<th></th>
<th>Severity</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R</strong></td>
<td><strong>P</strong></td>
<td><strong>R</strong></td>
</tr>
<tr>
<td>Physical functioning</td>
<td>-0.421</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Role/social–emotional/behavioral</td>
<td>-0.332</td>
<td>0.006</td>
</tr>
<tr>
<td>Bodily pain</td>
<td>-0.460</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Role/social–physical</td>
<td>-0.364</td>
<td>0.002</td>
</tr>
<tr>
<td>Family activities</td>
<td>-0.272</td>
<td>0.026</td>
</tr>
<tr>
<td>Physical summary score</td>
<td>-0.417</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

with severity (Table 3). In addition, scores in the physical functioning (p = 0.004) and bodily pain (p = 0.001) categories, as well as the Phs (p = 0.006), were significantly associated with treatment type.

Nine CHQ-PF50 domains (physical functioning, role/social–physical, bodily pain, general health perception, role/social–emotional/behavioral, mental health, parent impact–time, family activities, and Phs) were significantly affected as assessed by the Kruskal-Wallis test, and were submitted to the multivariable analysis. Among these domains, physical functioning, role/social–physical, role/social–emotional/behavioral, and Phs were affected by both scoliosis subtype and severity. The other domains were significantly influenced by scoliosis subtype only.

4. DISCUSSION

This cross-sectional study used the CHQ-PF50 to determine whether differences in the HRQoL existed among child and adolescent patients with different types of scoliosis. The results demonstrate that NMS was the most affected type, with reduced scores in both physical and psychosocial domains, followed by SS, IS, and CS.

Strength of the current study includes first applying of CHQ-PF50 in evaluation of patients with different type of scoliosis in both physical and psychosocial aspect. Limitations of the present study include, first, a relatively small number of study participants, which may have resulted in a selection bias. Furthermore, the NMS and SS groups represented various underlying diseases, and our sampling may not reflect the true composition of scoliosis subtypes in the natural population. Beyond the spine disease, accompanying conditions such as mental retardation and socioeconomic status were not examined, which would have required a larger sample size for accurate assessment.

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may have contributed to the observed differences in HRQoL. Additionally, the study was cross-sectional in nature. Several scoliosis-related factors not examined by our study may have affected the HRQoL (e.g., the age of onset, recent progression of the Cobb's angle, or curve type).

Overall, the scores for physical functioning, role/social–physical, role/social–emotional/behavioral, self-esteem, general health perception, family activities, and PHQ were lower in patients with scoliosis compared with those in healthy subjects. The general health perception was the most affected domain as reported by parents, followed in order by the family activities, self-esteem, role/social–emotional/behavioral, physical functioning, and role/social–physical domains.

Generally, observation rather than treatment is suggested for mild scoliosis, whereas watchful waiting, bracing, and surgery are suggested for moderate to severe scoliosis. In the present study, there were fewer surgery cases than patients with severe scoliosis, suggesting that the patients and their families preferred nonsurgical treatments. This might be related to cultural differences; Asians tend to be more concerned with and less willing to accept surgery. In a study examining patient ethnicity and treatment decisions, Asians had a lower preference for surgery compared with those in other ethnicities.30

While physical and psychosocial domains were both affected in NMS and SS, all of the physical and psychosocial domains, except self-esteem, general health and family cohesion, were affected in the NMS group. Considering the nature and severity of the underlying diseases, it is not surprising that there were more impacted HRQoL domains in patients with NMS or SS than in patients with IS or CS. Diseases such as CP, spinal dysraphism, and VACTERL association have been reported to significantly decrease the HRQoL relative to that in healthy subjects.31,32 Presumably, the comorbidities associated with the scoliosis may have dominated the HRQoL scores in the current study. Patients with CP, spinal dysraphism, or hydromyelia would experience a more profound impact physically, which may lead to reduced HRQoL. The heterogeneity of the SS group might account for the smaller reduction in the HRQoL compared with that in the NMS group; the HRQoL might be only mildly affected in patients with Marfan syndrome, whereas it may be more severely affected in patients with cerebro-costo-mandibular syndrome or cri-du-chat syndrome. Although the sampling of disease in NMS and SS may have led to some errors, there is a lack of large-scale studies that have investigated the prevalence of each syndrome among scoliosis subtypes. Most of the significantly affected categories in the IS group were psychosocial in nature. Using the SRS-22 questionnaire, Lee et al. observed that self-image was significantly decreased in AIS while the overall HRQoL was not significantly affected.13 Our similar results are not surprising given that appearance and body image are major concerns in the adolescent population. In addition, these results indicate that physicians should be more aware of this psychosocial issue. Not only should health education and communication with the patient and family be emphasized, but patients with IS should also be screened for potentially psychological diseases.

In the CS group, only the general health perception was significantly affected. Moreover, the CS group was the only group without affected family activities. Although there were no significant differences in age or Cobb's angle, patients with CS were slightly younger (mean age: 9.8 years) compared to those in the other three groups, which might explain this observation. Before adolescence, physical demands and expectations are lower, and self-image is not so important. Therefore, in patients with CS, the PS was affected to a lesser extent (ES = 0.12) than the PHS (ES = 0.60).

Based on the results in Table 2, one might conclude that the general health perception was negatively impacted in all types of scoliosis. However, the general health perception was affected the least in patients with IS and the most in patients with NMS. Bodily pain was only significantly affected in patients with NMS, which may have been confounded by factors such as spasticity in CP or joint contracture caused by neuromuscular disease.

The present results also suggest that treatment type and severity correlate with certain HRQoL domains, mainly those in the physical construct. Thus, psychosocial issues in the domains significantly affected in the present survey, such as role/social–emotional/behavioral and family activities, should be emphasized regardless of sex, treatment type, or severity. Psychological support and possible referrals for all patients with scoliosis are suggested for a comprehensive approach. In addition, the multivariable analysis confirmed that both the subtype and scoliosis severity affected the HRQoL in certain domains, and that the type of scoliosis appears to have more impact. Therefore, special medical attention, in terms of related physical and psychosocial issues, is highly recommended when caring for patients with NMS/SS and severe scoliosis in clinical practice.

In conclusion, the current study used the CHQ-PF50 questionnaire to evaluate differences in the HRQoL among four types of scoliosis in children and adolescents. Among the four types, the HRQoL was most affected in patients with NMS, with significantly lower scores in both physical and psychosocial domains compared with those in healthy controls. To a lesser extent, patients with SS were affected in both physical and psychosocial domains. In patients with IS, role/social–emotional/behavioral, self-esteem, general health perception, and family activities scores were lower than those in healthy controls. In contrast, patients with CS were only affected by decreased general health perception scores. Treatment type and severity correlated with the HRQoL. Although the scoliosis subtype and severity both affected the HRQoL, the scoliosis subtype demonstrated a greater influence. Special medical attention in certain physical and psychosocial issues should be considered and integrated into clinical practice.

ACKNOWLEDGMENTS

This study was supported by Yen Tjing Ling Medical Foundation, grant number C1-103–23. We thank the Biostatistics Task Force of Taipei Veterans General Hospital for the statistical assistance.

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