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Expressions of miRNAs in Papillary Thyroid Carcinoma and Their Associations with the BRAF^{V600E} Mutation and Clinicopathological Features.

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Objectives: The microRNAs (miRNAs) are known to be commonly expressed in papillary thyroid carcinoma. The BRAF^{V600E} mutation is the most common genetic mutation in thyroid cancer. The main aim of this study was to determine the possible association between expression of the three miRNAs and that of BRAF^{V600E} mutation and the clinicopathological features in papillary thyroid carcinoma.
Methods: This study was conducted on 51 paraffin-embedded tissues (42 thyroid cancer, 9 benign tumor) obtained from patients undergoing thyroidectomy at the Endocrine Center of OOO University Hospital.
Results: miRNAs expression was significantly high in patients with cervical lymph node metastasis and advanced TNM stage. In addition, miR-146b expression levels were significantly higher in papillary thyroid carcinoma patients with BRAF^{V600E} mutation. The relative quantification ($2^{-\triangle\triangle Ct}$) of miR-146b was also high among the miRNAs. Individually, the AUCs for miRNA-146b was 0.923 (cutoff value -1.97, sensitivity 88.9%, specificity 85.7%).
Conclusions: Especially, expression of miR-146b increased higher in PTC patients with BRAF^{V600E} mutation. These findings showed a role of miR-146b as potential biomarkers in differentiating PTC from benign tumor and as a prognostic indicator of PTCs. Further investigation will need for the roles of miRNAs in the pathogenesis of papillary thyroid carcinomas.

Key Words: BRAF^{V600E} mutation, MicroRNA, Papillary thyroid cancer

Thyroid cancer is the most common type of endocrine malignancy, accounting for approximately ≥ 92% of all endocrine malignancies, with the fastest worldwide growth in occurrence rate over the past few years. Papillary cancer is the most common among thyroid cancers, accounting for approximately 80% – 95% of thyroid cancers, most of which are papillary microcarcinomas.[1] Papillary carcinoma exhibits relatively slower growth compared to cancers occurring in other tissues and is known to display a rather positive prognosis. However, as 30% – 50% of the papillary carcinoma cases exhibit cervical lymph node metastasis at the time of diagnosis and 5% can be life-threatening with accompanied distant metastasis, the prediction

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of aggressive papillary carcinoma prior to surgery could assist in the planning of treatment.[2] The clinicopathological features used to predict aggressiveness include the age of the patient at the time of diagnosis, the stage of papillary carcinoma, tumor size (≥ 3 cm), extrathyroidal extension, lymph node metastasis, distant metastasis, insular variant, tall cell variant, and diffuse sclerosing variant; cases demonstrating these features are known to show poor prognosis.[2] The presence of biomarkers that can predict the aggressiveness of the tumor could, in addition to these clinical features, function as potential targets for the diagnosis and treatment of papillary carcinoma.

The $BRAF^{V600E}$ mutation is the most common genetic mutation in thyroid cancer and is particularly common in papillary carcinoma and anaplastic carcinoma developing from papillary carcinoma; however, it has not been observed in follicular or medullary carcinoma.[3] The frequency of occurrence of the $BRAF^{V600E}$ mutation has been reported in the range of 30% – 83%; however, countries having high iodine consumption, such as South Korea, have shown a higher frequency of occurrence.[4,5] The $BRAF^{V600E}$ mutation regulates the division and proliferation of thyroid cells through the mitogen activated protein kinase (MAPK) pathway and is involved in carcinogenesis through interactions with rearranged during transfection/papillary thyroid carcinoma (RET/PTC) and mutations in the rat sarcoma viral oncogene homolog (RAS).[6] The $BRAF^{V600E}$ mutation has been reported to show high-risk clinicopathological features, as it is related to lymph node metastasis, extrathyroidal extension, and relapse.[7] Conversely, another report has indicated that the $BRAF^{V600E}$ mutation is unrelated to the prognosis of papillary thyroid carcinoma.[8]

MicroRNA (miRNA) is a single stranded non-coding RNA with 21 – 25 nucleotides. It is transcribed from DNA as a pri-mRNA, and undergoes various processes, such as endonucleolytic cleavage, nuclear export, and strand selection, in order to be transformed into mature miRNA.[9] The mature miRNA generated in this manner forms RNA-induced silencing complexes (RISC) in order to suppress the expression of target genes, functioning as a negative regulator of vital cellular activities, such as cellular proliferation, differentiation, and apoptosis.[10] Currently, over 1,000 miRNAs have been discovered in humans, and the targets of over 1/3 of these miRNAs have, in turn, been discovered.[11] These miRNAs function as tumor suppressors or oncogenes in various cancers.[12,13] miR-21, miR-31, miR-34a, miR-122a, miR-146b, miR-155, miR-187, miR-204, miR-205, miR-221, and miR-222 are expressed in papillary thyroid carcinoma; among these, the expression of miR-221, miR-222, and miR-146b has been reported to be distinctly elevated, compared to normal tissues.[13,14] Studies investigating the association between miRNA and the $BRAF^{V600E}$ mutation in thyroid cancer are rare. Nikiforova et al.[15] and Cahill et al.[16] reported that the expression of miR-127, miR-130a, miR-141, miR-144, miR-146b, miR-155, miR-187, miR-200a, miR-200b, miR-221, and miR-222 was dysregulated in thyroid cell strains expressing the $BRAF^{V600E}$ muta-

tion. Meanwhile, Chou et al.[17] reported that, in addition to the expression of miR-221, miR-222, and miR-146b, the high risk group of papillary thyroid carcinoma (displaying the $BRAF^{V600E}$ mutation) demonstrated elevated expression of miR-221 and miR-146b and a significant elevation in miR-146b expression; a follow-up study also indicated that miR-146b increased the mobility and invasiveness of cancerous thyroid cells, and increased their resistance to chemotherapy-induced apoptosis.[18]

This study aimed to investigate the possible clinicopathological relationships between miR-221, miR-222, and miR-146b expression and the $BRAF^{V600E}$ mutation in papillary thyroid carcinoma.

METRIALS AND METHODS

Subjects

This study was conducted on portions of paraffin-embedded tissues obtained from patients who had undergone thyroidectomy at the Endocrine Center of the Kosin University Gospel Hospital. Tissues were obtained from a total of 51 cases; among these, 42 cases involved thyroid cancer and 9 cases involved benign tumor. The mean age of the patients was 47.1 ± 11.2 years (range 28 – 75 years), and the male to female ratio was 1:4.3. This study was approved by the Institutional Review Board of Kosin University Gospel Hospital.

Methods

1) Classification of risk groups

The American Joint Committee on Cancer Staging System for differentiated cancers published in 2016[19] was used to classify patients who were 55 years or younger and had TMN stage 1 or were older than 55 years and had TMN stage 1 or 2 into the low-risk group. The remaining patients were classified into the high-risk group.

This study has been approved by the Institutional Review Board of the Kosin University Gospel Hospital (approval no. 2014-10-140).

2) $BRAF^{V600E}$ mutation analysis

(1) DNA extraction

Genomic DNA was extracted using the QIAamp DNA FFPE Tissue kit (Qiagen, Hilden, Germany), from paraffin-embedded tumor tissues that were cut to a thickness of 10 μm and were obtained from patients subjected to thyroidectomy.

(2) Amplification

The DNA was amplified by adding 50 ng of DNA to a mixture containing 10 μL 2X concentrated HotStarTaq Master Mix (Qiagen), 3 mM $MgCl_2$, 0.3 μM primer pairs, and 400 μM deoxynucleotide triphosphate. The amplification of BRAF exon 15 was performed using the forward and reverse Exon 15 primers: forward, 5′-atgcttgctctgataggaaaatga-3′; reverse, 5′-agcagcatctcagggcca-3′. The PCR reaction conditions were set as follows: 35 repeats of 30 s at 94°C, 30 s at 58°C, and 45 s at 72°C, and a final extension for 10 min at 72°C.

(3) Direct sequencing

DNA was purified from a slice of the 2% agaro-

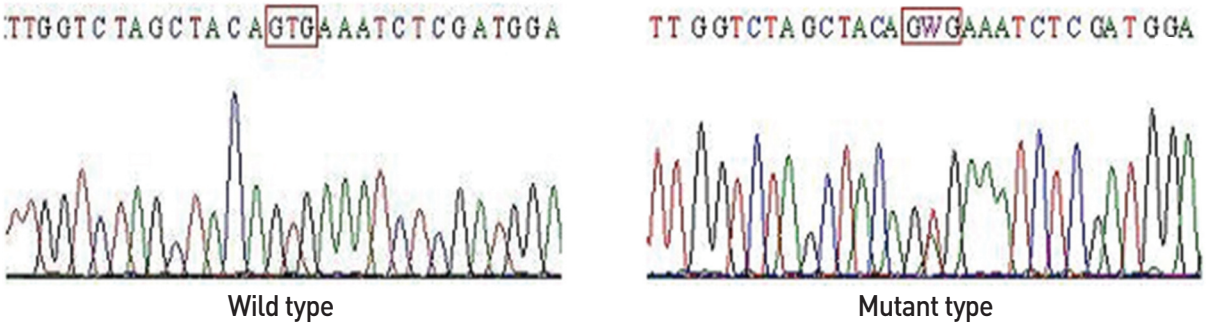

Fig. 1. Wild-type and mutant sequences of codon 600 of the BRAF gene.

gel (used for electrophoresis) using the QIAgen gel extraction kit (Qiagen). The purified product was amplified using fluorescence-activated ddNTP (BigDye3.1; Applied Biosystems, Foster City, CA, USA); subsequently, ethanol was used for purification. The purified product was dissolved in 10μL HiDi-Formamide, and the solution analyzed using an ABI PRISM 3100 automated capillary DNA sequencer (Applied Biosystems) (Fig. 1).

3) Expression of miRNA-146 b, miRNA-221, and miRNA-222

(1) RNA extraction

Total RNA was separated from the paraffin-embedded tissues; the paraffin tissue was placed in an RNase-free tube to which 300 μL melting buffer was added. This was centrifuged and incubated at 72°C for 10 min. Subsequently, 20 μL of proteinase K solution was added to this solution. The extract was mixed with 400 μL of binding buffer and 800 μL of 100% ethanol at 60°C. Following this, a cartridge was placed within the collection tube in order to transfer 700 μL of the extract containing the binding buffer and ethanol; this was then centrifuged twice. Five hundred microliters of the wash buffer and 100% ethanol were added to the cartridge and centrifuged; this process was performed in triplicate, and the final solution discarded. RNAse-free DW (50 μL) at 65°C was added to the cartridge; the tube was incubated for 1 min, and subsequently centrifuged. The total RNA remaining in the tube following the removal of the cartridge was used in further experiments.

(2) Quantitative real time polymer chain reaction (qRT-PCR)

The miR-221, miR-222, and miR-146b microRNA expression was measured using the TaqMan miRNA assay. Two microliters of the synthesized cDNA was subjected to quantitative real time PCR, using the ABI PRISM 7000 sequence detection system (Applied Biosystems). The cDNA was mixed with a 20 μL solution containing 10 μL TaqMan 2X universal PCR master mix (Applied Biosystems), 1 μL of the primer and probe mix (20X), and 7 μL RNase free water. As a double analysis, the level of miRNA expression was measured. U6 small RNA (RNU6B) was quantified as the standardized control group for the target miRNA. The same sample was used to perform quantitative real time PCR three times, in order to obtain the mean value for quantifying the level of expression. The

threshold cycle value (Ct value) of each PCR reaction, generated using the RNU6B expression as a reference, was standardized. The standardized values were converted to relative values. The relative quantity of miRNA for each sample was calculated as △Ct, which was the value obtained by subtracting the Ct value of RNU6B (used as the endogenous control) from the Ct value of the sample. The relative quantification of gene expression between the malignant and benign tumors was calculated via the $2^{-\triangle\triangle Ct}$ method (Applied Biosystems user bulletin no. 2 (P/N 4303859)), using the *ΔΔ*Ct value (difference between the △Ct value exhibited by papillary thyroid carcinoma patients and the △Ct value of benign nodes).

Statistical analysis

The clinicopathological features of the subjects were demonstrated using the independent t-test and chi-square test. The changes in miRNA expression were displayed as mean ± standard deviation (SD). The differences in miRNA expression between benign and malignant nodes were compared by independent t-test, and the differences between the expression of various miRNAs were analyzed by one-way analysis of variance (ANOVA); a post-hoc test was performed using the Scheffe's method. A ROC (receiver operating characteristic) curve analysis was performed in order to assess the possibility of using miR-221, miR-222, and miR-146b as diagnostic biomarkers for papillary carcinoma. The SPSS (version 18.0; IBM, Armonk, NY, USA) software was used for statistical analysis, and the significance level was set to $P < 0.05$

. RESULTS

Clinicopathological features of papillary carcinoma

The mean tumor size observed in the 42 papillary carcinoma test subjects was 15.2 ± 9.9 mm; 23 of these cases (54.7%) tested positive for the $BRAF_{V600E}$ mutation. We also observed 22 cases (52.4%) of extrathyroidal extension, 22 cases (52.4%) of lymph node metastasis, 27 cases (64.3%) of low-risk TMN staging group, and 15 cases (35.7%) of high-risk TMN staging group (Table 1).

Association between miR-221, miR-222, and miR-146b expression and the clinicopathological features of papillary thyroid carcinomas

miR-221 expression was high in the lymph node metastasis ($P < 0.01$) high-risk TMN staging group ($P < 0.01$); in addition, miRNA-222 and miRNA-146b expression was significantly high in cases displaying a tumor size ≥ 1 cm ($P = 0.02$), extrathyroidal extension ($P<0.01$), lymph node metastasis ($P = 0.01$), and the high-risk TMN staging group ($P < 0.01$). In particular, miRNA-146b expression was significantly elevated in the group expressing the $BRAF_{V600E}$ mutation, compared to the group without the mutation ($P = 0.02$) (Table 2).

Comparing the miR-221, miR-222, and miR-146b expression in benign and malignant tumors

Table 1. Clinicopathogenic features of papillary thyroid carcinomas patients analyzed in this study

Clinicopathological features	Number
Age (years)	47.1 ± 11.2
Gender (male:female)	8:34
Tumor size (mm)	15.2 ± 9.9
$BRAF^{V600E}$ positive	23
Multifocality	
Single	24
Multiple	18
Tumor location	
Unilateral	30
Bilateral	12
Tumor subtype	
Classic	36
FVPTC	6
Extrathyroidal extension	22
Lymphovascular invasion	6
Nodal metastasis	
N0	20
N1a	11
N1b	11
pTMN staging	
I	27
III	9
IV	6

FVPTC; follicular variant papillary thyroid carcinoma

The mean △Ct values of miR-221, miR-222, and miR-146b in benign tumor cases were -0.11 ± 1.65, -1.65 ± 1.31, and -0.09 ± 1.61, respectively; on the other hand, the mean △Ct values of miR-221, miR-222, and miR-146b observed in papillary carcinoma cases were -1.75 ± 1.62, -4.17 ± 1.53, and -3.71 ± 1.98, respectively. The △Ct values for all 3 miRNA were significantly elevated in papillary carcinoma cases, compared to the benign tumor cases ($P < 0.01$) (Fig. 2).

In terms of the differences in expression between the different types of miRNA, miR-222 and miR-146b showed a significantly elevated expression compared to miR-221 ($P < 0.01$); however , no significant differences were observed between miR-222 and miR-146b (Fig. 2).

The relative quantification ($2^{-\triangle\triangle Ct}$) revealed that genetic (microRNA) expression was significantly higher in malignant tumors than in benign tumors ($P < 0.01$). The $2^{-\triangle\triangle Ct}$ values for miR-221, miR-222, and miR-146b expression in papillary carcinoma were 4.91 ± 8.31, 9.18 ± 20.14, and 23.13 ± 28.59, respectively; this indicated

Table 2. Differences in miRNA expression in papillary thyroid carcinoma patients displaying different clinicopathological features

Clinicopathologic features (n = 42)	miR-221		miR-222		miR-146b	
	-ΔCT[a]	*P*-value	-ΔCT	*P*-value	-ΔCT	*P*-value
Age						
< 55 years (n = 18)	1.36	0.18	3.78	0.16	3.59	0.73
≥ 55 years (n = 24)	2.04		4.45		3.79	
Gender						
Male (n = 8)	2.31	0.28	4.62	0.36	4.83	0.06
Female (n = 34)	1.61		4.06		3.44	
Tumor size						
≤ 10 mm (n = 15)	7.61	0.02	3.21	0.02	2.17	0.00
> 10 mm (n = 27)	2.29		4.69		4.56	
Capsular invasion						
No (n = 20)	7.52	0.00	3.34	0.00	2.62	0.00
Yes (n = 22)	2.65		4.92		4.69	
Multifocality						
Single (n = 24)	1.08	0.00	3.78	0.05	3.37	0.19
Multiple (n = 18)	2.94		4.68		4.14	
$BRAF^{V600E}$ mutation						
Negative (n = 19)	1.56	0.49	3.98	0.47	2.94	0.02
Positive (n = 23)	1.90		4.32		4.34	
Nodal metastasis						
Negative (n = 20)	1.04	0.00	3.56	0.01	2.65	0.00
Positive (n = 22)	2.38		4.69		4.66	
Tumor staging[b]						
Low risk (n = 27)	1.08	0.00	1.09	0.00	3.06	0.00
High risk (n = 15)	2.94		2.94		4.86	

All data have been presented as mean values.

[a] $-\Delta C_T = (Ct_{miRNA} - Ct_{U6})$.

[b] The low-risk group was defined as comprising patients who were younger than 55 years and had stage I PTC and those aged 55 years or greater with stage I or II PTC, according to the AJCC. The remaining patients were included in the high-risk group.
miRNA, micro RNA; Ct, threshold cycle.

that the $2^{-\triangle\triangle Ct}$ value of miR-146b was significantly higher than that of miR-221 or miR-222 ($P<0.01$). However, no significant differences were observed between miR-221 and miR-222 (Fig. 3).

ROC curve analysis of miR-221, miR-222, and miR-146b expression in benign tumor and papillary carcinoma

The diagnostic value of miR-221, miR-222, and miR-146b for papillary thyroid carcinoma was assessed by performing an ROC curve analysis. miR-221 showed an area under the curve (AUC) of 0.762 (95% CI; 0.591 – 0.932), a cutoff value of -0.93, and a sensitivity and specificity of

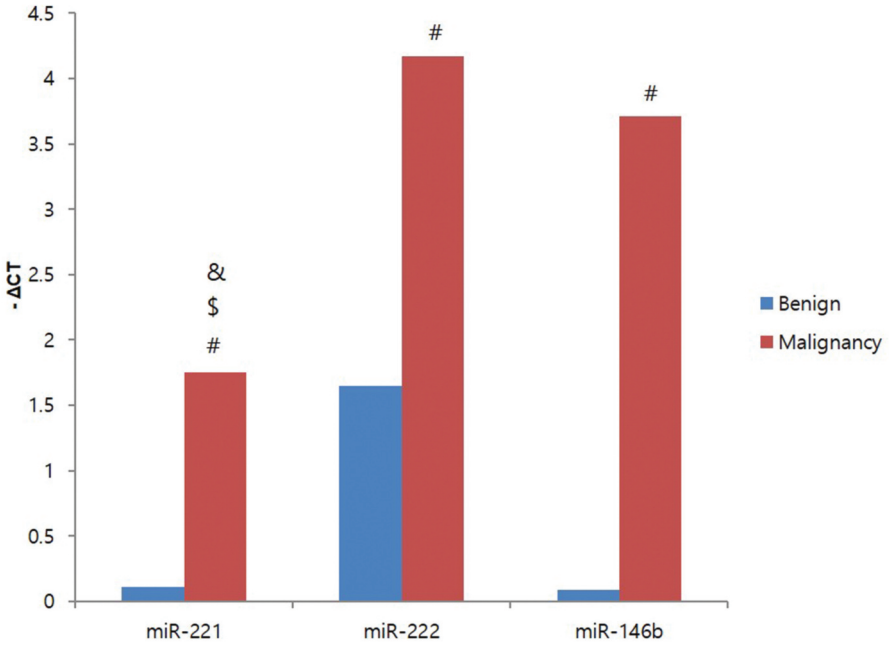

Fig. 2. Levels of expression of miRNA in patients with papillary thyroid carcinoma (n = 42) and benign tumor (n = 9). # $P<0.01$, as determined by the independent T-test, between the expression levels of miR-221 and miR-222 and miR-146b in papillary thyroid carcinoma and paired benign tumor. $ $P<0.01$ by ANOVA between the expression levels of miR-221 and miR-222. & $P<0.01$ (ANOVA) between the levels of expression of miR-221 and miR-146b.

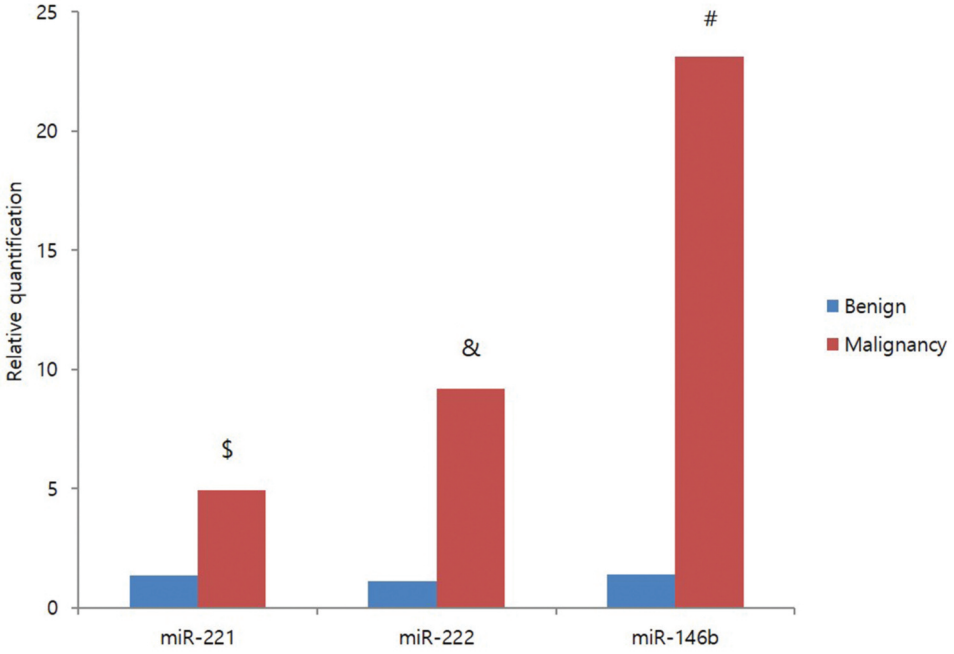

Fig. 3. Relative quantification of miR-221, miR-222, and miR-146b in patients with papillary thyroid carcinoma or benign tumor. # $P<0.01$, as quanti fied by independent T-test, between the levels of miR-146b in papillary thyroid carcinoma and paired benign tumor. $ $P<0.01$, when the expression of miR-221 and miR-146b were compared by ANOVA. & $P<0.01$, when the levels of expression of miR-222 and miR-146b were compared by ANOVA.

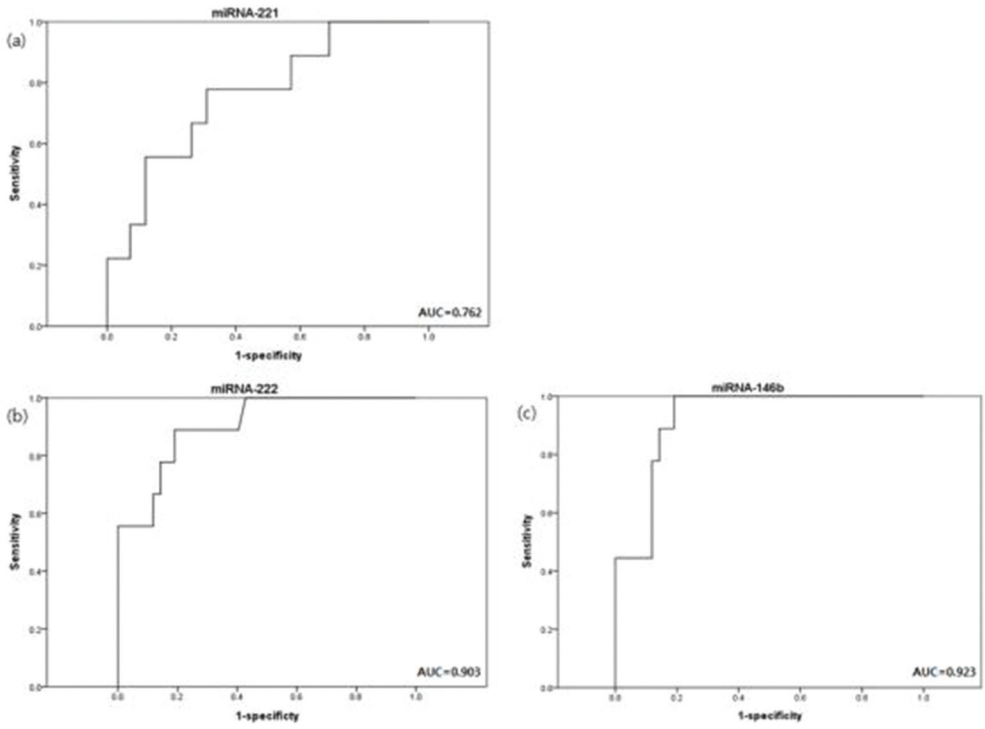

Fig. 4. ROC curve analyses of (a) miR-221, (b) miR-222, and (c) miR-146b to help discriminate the patients with papillary thyroid carcinoma from those with benign tumor. The AUCs for miR-221, miR-222, and miR-146b were 0.762, 0.903, and 0.923, respectively.

77.8% and 69.0%, respectively. miR-222 showed an AUC of 0.903 (95% CI; 0.803–1), cutoff value of -3.03, and sensitivity and specificity of 88.9% and 81.0%, respectively. miR-146b showed an AUC of 0.923 (95% CI; 0.849–0.998), a cutoff value, sensitivity, and specificity of -1.97, 88.9%, and 85.7%, respectively (Fig. 4).

DISCUSSION

In this study investigating the miRNAs miR-221, miR-222, and miR-146b, and $BRAF^{V600E}$ mutation (whose expression is commonly enhanced in papillary thyroid carcinoma), there was a significant increase in all miRNA in papillary carcinoma, compared to the benign tumor. The study conducted by Sun et al.[20], which investigated miR-221, miR-222, miR-146b, miR-181, and miR-21, reported a significant increase in the miR-221 and miR-222 expression in the lymph node metastasis and high-risk TMN staging group. Chou et al.[17] demonstrated that, although the expression of the miRNAs miR-221, miR-222, and miR-146b were elevated in the high-risk TMN staging group, no differences were seen between the patients with or without lymph node metastasis. In this study, all three miRNAs, miR-221, miR-222, and miR-146b, showed high levels of expression in the lymph node metastasis and high-risk TMN staging group; miR-222 and miR-146b expression was significantly increased in the extrathyroidal extension, with a tumor size ≥ 1 cm. This demon-

strated the association of miRNA expression with the clinicopathological features of tumors.

With the increase in prevalence of ultrasound testing, a greater number of cases of thyroidal nodules are being discovered; fine needle aspiration cytology is used in the diagnosis of these thyroidal nodules. However, shortcomings such as insufficient specimens, unconfirmed diagnosis, and/or false negative results have led to the demand for a new diagnostic method.[21] Gene detection technology has advanced significantly over the past 10 years. This has allowed for the analysis of tumorigenesis and progression of papillary thyroid carcinoma at a molecular biology level, as well as the development of biomarkers for diagnosis and prediction papillary thyroid carcinoma prognosis. Among the currently available genetic technologies, specific miRNAs (and their expression) can be applied as potential molecular biology biomarkers for papillary thyroid carcinoma. Sun et al.[22], who used 5 miRNA (miR-221, miR-222, miR-146b, miR-181, and miR-21) expressed in thyroid tumors, reported that miRNA-146b had the highest diagnostic value, with sensitivity, specificity, and AUC of 90.4%, 88.9%, and 0.952, respectively. This demonstrated its potential use as a potential biomarker in order to discriminate between benign and malignant tumors. Our study also showed miRNA-146b to have the highest diagnostic value with 88.9% sensitivity, 85.7% specificity, and an AUC of 0.923. Moreover, the relative quantification ($2^{-\triangle\triangle Ct}$) of genetic expression between benign and malignant tumors revealed that miR-146b expression was higher than that of miR-221 or miR-222; in addition, the positive results for the $BRAF^{V600E}$ mutation were also related to miR-146b. Chou et al.[18], based on the results of a multivariate logistic regression analysis, reported that in addition to being an indicator of cervical lymph node metastasis and the stage of tumor, miR-146b expression was an independent risk factor indicating the poor prognosis of papillary carcinoma. Furthermore, higher miR-146b expression levels were associated with a notably lower overall survival rate, compared to cases with lower levels of expression; the hazard ratio was also 3.92 times higher.

The target genes regulated by miR-146b, and the molecular mechanism by which miR-146b influences the aggressiveness of tumor cells in papillary carcinoma are not well known. However, Geraldo et al.[22] reported that miR-146b induced the expression of SMAD4 in order to regulate the signal pathway of transforming growth factor (TGF)-β in thyroid tumorigenesis; additionally, they stated that the over-expression of miR-146b in PCCL3 cells triggered cell proliferation even in the absence of thyroid stimulating hormone (TSH). In addition to papillary thyroid carcinoma, miR-146b is expressed in other solid tumors. The expression of the BRCA1 gene in a breast cancer cell line was down-regulated by miR-146b, resulting in increased cell proliferation.[23] miR-146b is also associated with poor prognosis in oral squamous cell carcinoma.[24]

The $BRAF^{V600E}$ mutation is capable of propagating tumorigenesis in the thyroid of transgenic mice[25]; this mutation was responsible for the increase in the invasion of thyroid cells in a study

using a thyroid cell line.[26] Temporary injection of small interfering RNA (siRNA) into a papillary carcinoma cell line with the BRAF mutation (to suppress BRAF) led to the suppression of cell growth and proliferation.[27] Although very few studies have been conducted on the BRAF mutation and miRNA expression in papillary thyroid carcinoma, the results of these were shown to be contradictory. Chou et al.[17] reported that the BRAFV600E mutation resulted in over-expression of miR-146b; Yip et al.[28] also stated that papillary thyroid carcinoma displaying the $BRAF^{V600E}$ mutation led to the over-expression of miR-146b, which was in turn associated with aggressive behavior. Our study also observed the over-expression of miR-146b in the group displaying the $BRAF^{V600E}$ mutation. On the other hand, Sheu et al.[29] stated that the $BRAF^{V600}$E mutation and expression of the miRNA set (miR-221, miR-222, miR-146b, miR-181, and miR-21) were unrelated to each other. In cases with the $BRAF^{V600E}$ mutation, a study conducted by Sun et al.[20] revealed significantly high expression of miR-221, miR-222, miR-146b, and miR-181, while another study by Huang et al.[30] revealed high expression of miR-21 and miR-203; however, these were attributed to the differences in specimens and examination methods.

In conclusion, miR-221, miR-222, and miR-146b showed high levels of expression in the lymph node metastasis and high-risk TMN staging group in our study; therefore, miRNA expression was associated with the clinicopathological features of this tumor. In addition, miR-146b showed the highest sensitivity and specificity among the three miRNAs. Moreover, miR-146b expression was demonstrated to be significantly increased in the presence of the $BRAF^{V600E}$ mutation; therefore, miR-146b was believed to be a potential biomarker for the diagnosis of papillary carcinoma. Based on this study, the mutual association between the $BRAF^{V600E}$ mutation and miRNA must be studied further, in order to determine the causes and progression of papillary carcinoma.

This study was conducted to examine the probable association between expression of miR-221, miR-222, and miR-146b and that of the $BRAF^{V600E}$ mutation and the clinicopathological features in papillary thyroid carcinoma. miR-221, miR-222, and miR-146b expression was high in the lymph node metastasis and high-risk TMN staging group, which indicated the association of miRNA expression with the clinicopathological features. All miRNAs showed significantly elevated expression in papillary carcinoma compared to benign tumor, with miR-221 showing the lowest level of expression among the miRNAs. miR-146b exhibited the highest sensitivity (88.9%), specificity (85.7%), and AUC (0.923), and was believed to show the most diagnostic value as a potential biomarker for differentiating between benign and malignant tumors. Moreover, miR-146b expression was significantly elevated in the presence of the $BRAF^{V600E}$ mutation. This study indicated the potential of miR-146b to be used as a biomarker for diagnosis of papillary carcinoma (in the future).

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Influences of Socioeconomic Status on Short Stature in Childhood

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Objectives: Short stature in childhood is defined to the cases in which the stature is below 3 percentiles of the standard value in accordance with that of those in the same age and gender group. The influence of the socioeconomic status on the short stature in childhood are analyzed.

Methods: 154 children from the community child center in a region of poor socioeconomic status and 78 children in normal socioeconomic status who visited the Busan Medical Center due to the issue of short stature were selected for examination and analysis.

Results: The prevalence rate of short stature at the community child center in 2 municipalities in Busan was confirmed to be 7.3%. In the comparison of the average growth parameters of poor socioeconomic status and normal socioeconomic status in the short stature group, there was no observation of significant difference in terms of the chronological age, mid-parental height, bone age, bone age/chronological age, height standard deviation score (SDS), body mass index(BMI) percentile and insulin like growth factor binding protein 3 (IGFBP3) SDS. In the short stature suspicious group, there was observation of significant difference in the averages of bone age, weight, BMI percentile , IGFBP3 and IGFBP3 SDS.

Conclusions: Although the prevalence rate of short stature in children belonging to the poor socioeconomic class was observed to be higher than the existing results, there was no significant difference in the growth parameters associated with the growth of the height from those of the children in normal socioeconomic status.

Key Words: Growth, Height, Short stature, Socioeconomic status

It has been reported that the growth of human beings is affected by a diverse range of factors including genetics, race, weight at the time of birth, hormone, nutrition and environment.[1,2] Although genetics is the most important factor as the decisive factor for the growth of height, it is also known that socioeconomic status and diseases also have influence. Globally, there had been increase in the average height of people by 1-3cm for every 10-year interval during the 20th century due mainly to improvement of the health, and advancement of environment and socioeconomic status of the children.[3-6] Although reports of socioeconomic difference affecting the growth of height have declined a lot in recent years, there have been reports that there are still influence.[5,7] Majority of children at the community child centers in Korea are the children of

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families subjected to the payment of basic living subsidies under the medical classification, families of lower income class, families of social class in health insurance blind zone and, socially, families of single parent and multi-child family. Therefore, there is a report that the frequency of short stature is high for the children in the community child center since they belong to socioeconomically vulnerable medical class.[8] However, there is insignificant study on the influence of socioeconomic status on short stature and growth of height in Korea. Accordingly, we measured the growth parameters such as height, weight, body mass index (BMI), midparental height, bone age and insulin like growth factor binding protein 3 (IGFBP3) of children with short stature detected during health checkup at the community child center and children visiting the short stature clinic of the pediatrics and adolescents department to determine the effects of socioeconomic status on the height growth and the frequency of short stature through comparison of the forementioned 2 groups.

MATERIALS AND METHODS

Subjects

This was a retrospective study conducted following approval from public institutional review board. Pediatrics and Adolescents Department and Public Medical Service Department of Busan Medical Center executed health examination on 582 children at the community child center in 2 municipalities in Busan Metropolitan City from January 1, 2014 to August 31, 2019. Among these children, a total of 154 children including 43 children with short stature who corresponds to below 3 percentile values of those in the same gender and age and 111 children who underwent examination at the short stature clinic of the pediatrics and adolescents department with suspicion of short stature by belonging to values from 3 percentile to 20 percentile were selected as the subjects of the study. At the same time, 78 children in the normal socioeconomic status who were treated for the diagnosis of short stature under the same aforementioned standards and due to the suspicion of short stature by belonging to values from 3 percentile to 20 percentile values at the Pediatrics and Adolescents Department of Busan Medical Center over the same period were put in the control group.

Methods

Same personnel training on the method of measuring the height of the children measured the height and weight of the subject children and Korean National Growth Chart of children in 2007 was used as the standard for computation of height percentile value while the Korean National Growth Chart for children in Korea in 2018 was applied as the standard for children born in 2019. X-ray was taken on the non-dominant wrist and hand to measure bone age with the TW3 (Tanner-Whitehouse 3) method as the standard. BMI was computed by using the formula, weight(kg)/[height(m)]^2. Percentile for BMI was computed by using the Korean National Growth Chart of children in 2007. Mid-

parental height SDS was calculated based on the data corresponding to the age of 18.0, which is nealy closet to adult height. Blood test was executed to measure IGFBP3. IGFBP3 standard deviation score (SDS) was computed by making reference to the reference values for children and adolescents of Korea in 2012.[9] The heights of children actually measured were compared by computing their SDS by dividing the value obtained by subtracting the average value of the height of children in the same age and gender from the actually measured height of each child by the standard deviation (SD).

SAS statistics program was used for statistical processing of data obtained. Wilcoxon/Mann-Whitney test (normal approximation two-sided) and two sample t-test were used to compare the average values while multiple logistic regression analysis was used for analysis of growth factor related analysis in accordance with low socioeconomic status. All statistically data are determined to be significant if the *P*-value is less than 0.05.

RESULTS

Characteristics of subject children and prevalence rate of short stature in children of low socioeconomic status

A total of 232 children were composed of 121 boys and 111 girls. Among these, there were 154 children from the community child center with poor socioeconomic status while 78 children were from normal socioeconomic status. There were 58 children with short stature belong to below 3 percentile values accounting for 25% of the total, while 174 children had suspicion of short stature belonging to values from 3 percentile to 20 percentile accounting for 75 % of the total. The average age of male and female on the subject children was 10.52 ± 2.83 years and 10.2 ± 2.55 years. The prevalence rate of short stature in childhood at the community child center in the 2 municipalities of Busan was confirmed to be 7.3% during the study period, which is substantially higher than the prevalence rates of 0.9% and 1.4% indicated in the Korean national growth chart of children in 2007 and 2017, respectively (Table 1) (Table 2) (Fig. 1).

Comparison of growth parameters in accordance with presence of short stature

Independent sample t-test was used for comparison of the average of the short stature group and short stature suspected group in all the subject children of the study. Short stature group displayed statistically significant lower height, height SDS, midparental height SDS, bone age, weight, BMI percentile and IGFBP3 (Table 3).

Comparison of growth parameters in accordance with the socioeconomic status

The proportion of children with short stature group among the children of poor scocioeconomic status was higher than that of normal socioeconomic status (27.9 % vs. 19.2 %). However, there was no significant difference in the chi-square test (*P*-value = 0.149). Wilcoxon/Mann-Whitney test (normal approximation two-sided)

Table 1. Clinical characteristics of subjects

Charaoteristios	Mean values	
	Male	Female
Total Patients (n)	121	111
Age (yr)	10.52 ± 2.83	10.20 ± 2.55
Bone age (yr)	10.59 ± 2.49	9.74 ± 2.32
Bone age/Chronologioal age	0.98 ± 0.11	0.91 ± 0.09
Midparental height (om)	170.8 ± 4.1	158.5 ± 3.58
Midparental height SDS	-0.3 ± 0.5	-0.3 ± 0.48
Height (om)	135.0 ± 15.54	133.0 ± 12.79
Height SDS	-0.81 ± 0.5	-0.7 ± 0.45
BMI (kg/m^2)	18.03 ± 3.19	16.84 ± 2.68
BMI peroentile (%)	42.71 ± 27.68	33.52 ± 24.41
IGFBP3 (ng/ml)	4347.26 ± 1063.8	4459.0 ± 975.8
IGFBP3 SDS	0.41 ± 0.29	0.36 ± 0.26

Data expressed means ± standard deviation
SDS, standard deviation score; BMI, body mass index; IGFBP3, insulin like growth factor binding protein 3

Table 2. Prevalence of short stature in low socioeconomic status children - comparison with Korean National Growth Charts

Short stature	Our result	KNGC2007	KNGC2017
Year	2014.1-2019.8	2007	2017
Age (yr)	5-17	2-18	2-18
Location	Busan (yeon-je gu, busan-jin gu)	Whole country	Whole country
Number/Total number	43/582	68/7606	106/7606
Prevalence rate	7.3%	0.9%	1.4%

KNGC, Korean National Growth Charts

was applied for the comparison of children with short stature between the children from poor socioeconomic status and children from normal socioeconomic status since the number of normal children is small, while independent sample t-test was applied for the children with suspicion of short stature. Children with short stature showed no difference in growth parameters in accordance with the socioeconomic status. In children with suspicion of short stature belonging to values from the 3 percentile to 20 percentile, significant differences in the averages of weight (32.5 ± 10.6 vs. 36.1 ± 12.9, P = 0.045), bone age (10.1 ± 2.3 vs. 10.9 ± 2.28, P = 0.017), bone age/chronological age (0.93 ± 0.1 vs. 0.99 ± 0.1, P = 0.001), BMI percentile (36.6 ± 24.2 vs. 47.3 ± 29.7, P = 0.010), IGFBP3 (4661.4 ± 914.7 vs. 4238.9 ± 989.4, P = 0.005) and IGFBP3 SDS (0.5 ± 0.2 vs. 0.3 ± 0.3, P = 0.008) were observed (Table 4) (Fig. 1).

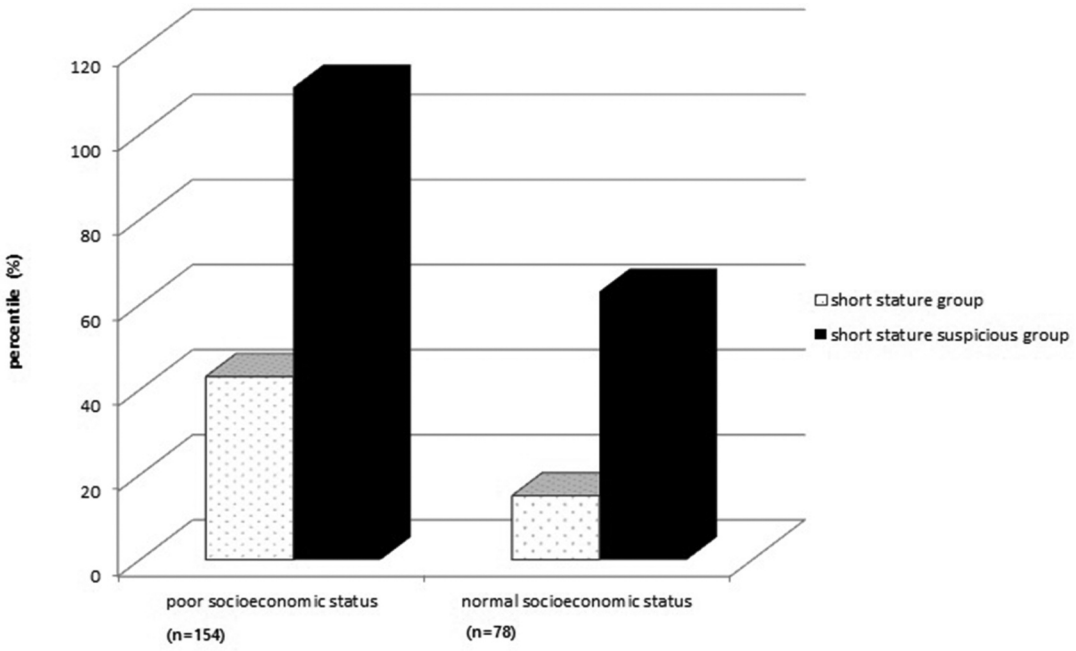

Fig. 1. Comparison between short stature group and short stature suspicious group according to socioeconomic status (chi-square *P*-value = 0.149)

Table 3. Comparison of clinical characteristics between short stature group and suspicious short stature group

variable	Short stature children(n = 58)	Suspicious short stature children(n = 174)	*P*-value
Chronological age (yr)	10.5 ± 3.1	11 ± 2.5	0.248
Bone age (yr)	9.6 ± 2.7	10.38 ± 2.33	0.031
Bone age/Chronological age	0.93 ± 0.1	0.95 ± 0.1	0.310
Midparnetal height (cm)	164.6 ± 7.5	165.0 ± 7.2	0.728
MPH SDS	-0.42 ± 0.58	-0.27 ± 0.46	0.045
Height (cm)	126.8 ± 15.1	136.5 ± 13.2	0.000
Height SDS	-1.4 ± 0.4	-0.6 ± 0.3	0.000
Weight (kg)	28.4 ± 12.2	33.8 ± 11.6	0.003
BMI (kg/m^2)	16.9 ± 3.2	17.7 ± 2.9	0.089
BMI percentile	31.9 ± 24.9	40.5 ± 26.8	0.033
IGFBP3 (ng/mL)	4077.7 ± 1134	4508.5 ± 961.4	0.005
IGFBP3 SDS	0.3 ± 0.3	0.4 ± 0.3	0.051

Short stature, defined as height ≦ 3 percentile; Suspicious short stature children, defined as 3 percentile < height ≦ 20 percentile
Data expressed means±standard deviation
MPH, midparental height; SDS, standard deviation score; BMI, body mass index; IGFBP3, insulin like growth factor binding protein 3

Multiple logistic regression analysis of factors associated with the diagnosis of short stature in accordance with the low socioeconomic status

Multiple logistic regression analysis was executed to confirm the growth parameters associated with the diagnosis of short stature in accordance with the low socioeconomic status. As the results, it was observed that BMI persentile (odds

Table 4. Comparison of clinical characteristics between poor socioeconomic status group and normal socioeconomic status group (in short stature children and suspicious short stature children)

variable	Short stature children		*P*-value	Suspicious short stature children		*P*-value
	low socioeconomic status group (n = 43)	Normal socioeconomic status group (n = 15)		low socioeconomic status group (n = 111)	Normal socioeconomic status group (n = 63)	
CA (years)	10.6 ± 2.7	10.1 ± 4	0.704	10.9 ± 2.5	11.2 ± 2.5	0.361
BA (years)	9.5 ± 2.5	9.8 ± 3.2	0.972	10.1 ± 2.3	10.9 ± 2.26	0.017
BA/CA	0.9 ± 0.1	1 ± 0.2	0.084	0.93 ± 0.1	0.99 ± 0.1	0.001
MPH (cm)	164.1 ± 7.78	166.13 ± 6.84	0.397	164.32 ± 7.45	166.28 ± 6.6	0.086
MPH SDS	-0.43 ± 0.62	-0.38 ± 0.4	0.846	-0.27 ± 0.46	-0.26 ± 0.46	0.846
Height (cm)	127.14 ± 13.26	125.85 ± 19.97	0.764	135.15 ± 12.56	138.8 ± 14.1	0.080
Height SDS	-1.4 ± 0.5	-1.3 ± 0.2	0.633	-0.7 ± 0.3	-0.6 ± 0.4	0.080
Weight (kg)	28 ± 9.6	29.4 ± 18.2	0.566	32.5 ± 10.6	36.1 ± 12.9	0.045
BMI (kg/m^2)	16.8 ± 2.7	17.3 ± 4.6	0.805	17.4 ± 2.8	18.1 ± 3.1	0.101
BMI percentile	29.7 ± 23.9	38.2 ± 27.3	0.253	36.6 ± 24.2	47.3 ± 29.7	0.010
IGFBP3	4176.1 ± 1047.5	3795.9 ± 1352.7	0.307	4661.4 ± 914.7	4238.9 ± 989.4	0.005
IGFBP3 SDS	0.3 ± 0.3	0.3 ± 0.4	0.602	0.5 ± 0.2	0.3 ± 0.3	0.008

Suspicious short stature children, defined as 3 percentile 〈 height ≦ 20 percentile
Data expressed means±standard deviation
MPH, midparental height; SDS, standard deviation score; BMI, body mass index; IGFBP3, insulin like growth factor binding protein 3

ratio, 0.982; 95% confidence interval, 0.97-0.995; $P = 0.005$), IGFBP3 (odds ratio, 1.001; 95% confidence interval, 1-1.001; $P = 0.0002$) and bone age/chronological age (odds ratio, 0.005; 95% confidence interval, < 0.001 - 0.118; $P = 0.001$) are associated with the possibility that all the subjected children would have low socioeconomic status (Table 5).

DISCUSSION

Although the height growth of child is generally determined genetically, it is also known to be influenced substantially by the daily life environment during early childhood. Factors that affect growth include nutrition, disease, psychosocial stress, residential conditions and physical damages during childhood that are difficult to bear with.[3,10,11] Such socioeconomic inequalities in height growth are observed consistently within variously different environments and there is the tendency of the finally predicted height becoming even smaller depending on the extent of the environmental damages confronted with such as malnutrition or aggravation of health by children with socioeconomic disadvantages. Final predictable height reflects not only genetic possibilities but also the daily life status during childhood.[3,5] In spite of the improvement in the standard of living and general increase in the height, there is a trend of children with poorer background displaying slower height growth and it is known that the difference in height continues

Table 5. Stepwise multivariate logistic analysis of factors associated with poor socioeconomic status

variable	*P*-value	Odd ratio (95%CI)
BMI percentile	0.005	0.982(0.97 - 0.995)
IGFBP3	0.0002	1.001(1 - 1.001)
Bone age/Chronological age	0.001	0.004(< 0.001 - 0.118)
Midparental SDS	0.684	0.872(0.45 - 1.68)
Height SDS	0.564	0.768(0.314 - 1.87)
short stature group vs. suspicious short stature group	0.498	1.396(0.533 - 3.657)

BMI, body mass index: SDS, standard deviation score

due to socioeconomic inequality.[12] In the researches that have been reported thus far, the effects of environmental factors on the height growth is decreasing in the recent years and similar research results are being reported for various other population groups. It is asserted that this is due to improvement in the socioeconomic situation.[13-15] Therefore, it is claimed that the correlation between the socioeconomic status and height growth of children is weakening and the social inequality in height is increasingly decreasing.[3,14] However, in the survey made in France over the last 30 years in another study, it is reported that there continues to exist social inequalities in the height growth and the final adult height due to factors such as education and income.[7] In domestic research, although the study was limited to the Seoul area, no significant difference was found in studies examining the height of high school students according to parent income.[16] But there was a report of 13% with short stature prevalence rate in children of married immigrant women in rural areas.[17] Influence of socioeconomic status on short stature is not yet consistent.

This study aimed to examine whether socioeconomic status influences the height growth of children by comparing the recent prevalence rate of short stature in the children of the community child center, who belong to the medically vulnerable status, and that of the children in normal socioeconomic status in Korea. During the period of this study, the prevalence rate of short stature in childhood at the community child center in 2 municipalities of Busan was observed to be 7.3%, which is substantially higher than the prevalence rates of short stature of 0.9% and 1.4% reported in the Korean National Growth Chart of children in 2007 and Korean National Growth Chart of children and adolescents in 2017, respectively.[18] The reason for the difference in prevalence rate may be because the Busanjin-gu and Yeonje-gu districts in Busan, which are the study areas, have high health deprivation indices in Busan.[19] Also, it is thought that the results of this study may be possible because there is also report of high prevalence rate of short stature in certain vulnerable groups.[17]

Although there could be limitations in reflecting the prevalence rate of short stature of children in all the community child center since it is based only on 2 specific municipalities in Busan, it nonetheless illustrates that socioeconomic status imparts influence on the height growth of children.

Although there are various factors that affect the growth status evaluation or growth velocity in children,[20] this study utilized midparental height, bone age, BMI and IGFBP3 for which confirmation of the results was possible. Midparental height is important for predicting the final height of children and is important for explaining the genetic factors of height growth. Midparental height is calculated by adding 6.5 cm from average height of parent in boys and subtracting 6.5 cm from average height of parent in girls.[16] Bone age is used for growth evaluation or predicting the final adult height of normal children. Bone age increases with the age and the height increase with the bone age.[21] BMI is known to impart different effects depending on the time and extent of height growth. Although it is known that the BMI and height growth has positive correlation within a period of less than 1 year, they are known to have negative correlation over a period of more than 1 year. In particular, it is asserted that BMI has negative effect on height growth in children with relatively short height.[22,23]

IGFBP3, which could be conducted in this study, is a test method capable of reflecting the concentration of blood growth hormone as an independent test and is used as a screening test at the time of growth evaluation of children. Although IGFBP3 is known to be less affected by external factors in comparison to IGF1 and is not affected by BMI, its normal value differs depending on the age and gender. As such, IGFBP3 SDS was applied in this study.[24-27]

For the comparison of the average of growth parameters between the short stature group and 3-20 percentile short stature suspected group among all the subject children in this study, it was confirmed that the short stature suspected group had higher bone age/chronological age, midparental height, midparental height SDS, BMI, and IGFBP3 SDS with no significant difference. Also, other growth parameters, the average values of bone age, height, height SDS, weight, BMI percentile, and IGFBP3, showed statistically significant high results in short stature suspected group. These results were consistent with previous reports.[27] In the comparison between the short stature group belonging to the below 3 percentile values who have poor socioeconomic status and the short stature group of the normal socioeconomic status, there was no statistically significant difference in the average values of all growth parameters between the 2 groups. In addition, in the results of comparison between the short stature suspected group children who have poor socioeconomic status belonging to values from 3 percentile to 20 percentile and the short stature suspected group of children from normal socioeconomic status, there was no observation of statistically significant difference in the average values of the growth parameters with the exclusion of bone age, bone age/chronological age, weight,

BMI percentile, IGFBP3 and IGFBP3 SDS. In the comparison of the socioeconomic differences both short stature group and short stature suspected group, there was no difference in midparental height and midparental height SDS.

Therefore the difference in height due to genetic factors could be excluded. On the contrary, IGFBP3 and IGFBP3 SDS were observed to be statistically significantly higher in the short stature suspected children from poor socioeconomic status childhood. This appears to be the result of greater number of girls at 61 in comparison to the boys at 50 in the children as well as the higher average age of children in the short stature suspected group with poor socioeconomic status belonging to values from 3 percentile to 20 percentile. Based on these results, it can be presumed that difference in the socioeconomic status does not impart any significant influence on the growth parameters of height growth except bone age and bone age/ chronological age in the short stature suspected group. In this study, factors related to the diagnosis of short stature in accordance with the socioeconomic difference were examined through multiple logistic regression analysis. As the results, BMI persentile and bone age/chronological age were found to have influence on the socioeconomic status, and IGFBP3 was also found to be a factor that imparts influence due to the difference in the normal value according to the age and gender. Although BMI persentile is associated with the growth velocity of height, it is presumed that this study has limitations in concluding that it affects socioeconomic status due to too large difference in accordance with the period of observation of sexual maturity and growth velocity of the height. In addition, it was found that other factors such as height SDS, midparental SDS and presence of short stature do not impart significant effect on the socioeconomic status. These results are in agreement of the results of numerous recent researches that the influence of the difference in the socioeconomic status on the height growth of children is declining.

As the results of this study, it was presumed that there is no significant difference in the growth parameters between the short stature children from poor socioeconomic status and from normal socioeconomic status. However, the prevalence rate of short stature children from poor socioeconomic status was confirmed to be higher than that of the Korean National Growth Chart for children, although limited to specific areas. Therefore, there is a need to check prevalence rate of short stature in children from the community child center in a broader range of areas. Also, this study has additional limitations. First, there were no growth parameters of height velocity over a specific period in the compared growth parameters. Second, there was no analysis of lifestyle habits, dietary habits and sleep habits that affect height growth. There is a need to conduct more extensive range of studies on greater groups for more diversified factors in order to discern the influence of socioeconomic status on the height growth of children.

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Influence of Cold Ischemia Time and Storage Period on DNA Quality and Biomarker Research in Biobanked Colorectal Cancer Tissues

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Objectives: Biobanking plays an important role in future research. Assessment and control of the preanalytical variables of biobanked tissues are fundamentals for the optimal use of biospecimens.

Methods: Forty-five colorectal cancer (CRC) tissues stored at -80℃ in Bio-Resource Bank were evaluated to define the influence of cold ischemia time (CIT) and storage period (SP) on DNA quality in biobanked tissues. Three CITs (less than 30 minutes (CIT-1), 30-45 minutes (CIT-2), and 45-60 minutes (CIT-3)) and three SPs (less than 1 year (SP-1), 2-3 years (SP-2), and 4-5 years (SP-3)) were chosen. NanoDrop spectrophotometer was used to determine the 260/280 ratio for DNA purity. DNA integrity was analyzed by a UV transilluminator following electrophoresis on 2% agarose gel. To evaluate the practical usability of DNA for biomarker research, KRAS mutation status was assessed by PCR amplification.

Results: All DNA specimens had a 260/280 ratio ranging between 1.8 and 2.0 with the exception of one specimen (CIT-2/SP-2 group). For DNA integrity, DNA appeared as a compact, high-molecular-weight band with no or scanty low-molecular-weight smears. The concordance of KRAS mutation status between paired biobanked frozen tissues and formalin-fixed paraffin-embedded tissues was 100%. DNA remained stable in CRC tissues kept at room temperature for up to 1 hour and long-term storage up to 5 years.

Conclusions: Storage conditions of our biobank are suitable for long-term (at least five years) specimen preservation with high DNA quality. These results have practical implications that could affect banking guidelines.

Key Words: Biobank, Colorectal cancer, DNA, Ischemia, Storage

High-quality biospecimens are necessary for biomedical research. Well-preserved frozen tissue is the favored biospecimen for biomedical research as it produces higher quality DNA, RNA, and proteins than formalin-fixed paraffin-embedded (FFPE) tissue.[1] Biobanking aims to systemically collect, transport, store, manage, and utilize fresh tissues from surgical removal for further projects under the standard operation procedures (SOP).[2] Therefore, biobanking plays an important role in clinical and translational research as well as future scientific research.

Preanalytical handling basically refers to all processes that occur until the analysis of a biospecimen.[3] Preanalytical variables including ischemia time (IT) and storage period (SP) can

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have significant effects on the integrity of the biospecimens and the results of downstream analyses.[3] In view of this, preanalytical variables must be precisely assessed and controlled in biobanking for optimal use of biospecimens in future.

Knowledge about acceptable cold ischemia time (CIT) (i.e., the time interval between surgical removal in the operating room and freezing of tissue at the pathology department)[4] or SP is considered essential to improve the quality of biospecimens. Previous studies reported conflicting results regarding DNA quality for long CIT or SP.[5-8] For colorectal cancer (CRC) tissues, a recent study reported minimal RNA change for up to 6 hours of CIT[9] and tissues stored in the liquid nitrogen tank for 11 years can provide high-quality specimens for medical research.[10]

Over the past decade, there have been significant advances in the molecular characterization of various cancers that are driving treatment decisions. In CRC patients, *KRAS* is one of the most important biomarkers for identifying patients who will respond to anti-epidermal growth factor receptor (EGFR) therapies.[11] In addition to *KRAS*, the number of molecular tests performed on CRC tissue specimen is constantly increasing. However, molecular diagnostics is confronted with its own specific challenges such as the amount of tissue specimens or archival status. Therefore, growing interest in molecular analysis in CRC can be facilitated by biobanked specimens.

Given the above considerations, the goal of this study was to define the impact of CIT and SP on DNA purity and integrity in biobanked CRC tissues. Furthermore, we assessed the concordance of *KRAS* mutation status between biobanked and FFPE tissues to evaluate the practical usability of DNA from a biobanked tissue for biomarker research. It is hypothesized that the results of this study will help to formulate appropriate guidelines for preanalytical processing of CRC specimens in biobanking.

MATERIALS AND METHODS

Biobanking of CRC tissue

Forty-five, fresh-frozen CRC tissues collected from 2013 to 2017 and stored at -80℃ in Bio-Resource Bank of Dong-A University Medical Center (DAMC) were evaluated in order to define the influence of CIT and SP on DNA quality and biomarker research in biobanked human CRC tissues. Handling of resected CRC tissues from patients was carried out by trained and experienced staffs according to the SOP of Bio-Resource Bank of DAMC. In short, resection specimens were transported (at room temperature without any conservation fluid) from the operating room to the pathology department, immediately following removal of the specimen from the patient. In the pathology department, the specimen was handled at room temperature and within 60 minutes after resection specimens were snap-frozen as described below. When the 60 minutes time limit was exceeded, no tissue samples were taken. Blood and necrotic tissues on the surface of the specimens were washed off

with pre-cooled phosphate-buffered saline (PBS). Subsequently, the tumor specimens were cut into small pieces (about 0.5 x 0.5 x 0.5 cm in dimension) under aseptic conditions. The frozen slides were observed by an experienced pathologist (M.S.Roh) and tumor specimens which comprised of ≥ 80% tumor nuclei and ≤ 20% necrosis, were considered as acceptable for future research. Tumors and normal tissues were repackaged in labeled cryovials and quickly frozen in -80℃ freezer with specialized temperature monitoring system to direct real-time temperature.

Study design

CRC tissues were chosen according to three CITs (less than 30 minutes (CIT-1), 31 - 45 minutes (CIT-2), and 46-60 minutes (CIT-3)) and three SPs (less than 1 year (SP-1), 2-3 years (SP-2), and 4-5 years (SP-3)). Next, the specimens were assigned to nine groups (Table 1) and five specimens were allocated to each group. In all cases, molecular analysis for *KRAS* mutation status was initially performed using FFPE tissue block at the time of pathologic diagnosis. The specimens used were collected after obtaining informed consent from the donors and immediately anonymized. The study protocol was approved by the Institutional Review Board of DAMC of Korea (DAUHIRB-18-099).

DNA preparation

Frozen tissues were prepared with optimal cutting temperature (OCT)-embedded tissue and cut into 5 μm pieces. The cut sections were washed with PBS and the OCT compound was removed. Cell lysis and protein removal were performed using a lysis buffer and proteinase K, respectively. DNA was extracted using the paramagnetic beads based Maxwell®16 Viral Total Nucleic Acid Purification Kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's instruction.

DNA purity analysis by spectrophotometry

After eluting DNA in 50 μl of elution buffer, the concentration and purity of extracted DNA were assessed using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The machine was calibrated and cleaned according to the manufacturer's instruction. Absorbance at 260 nm and 280 nm for 2 ㎕ of each DNA specimen was measured. The corresponding elution buffer was used as blank. Due to DNA absorbance at a wavelength of 260 nm and protein absorbance at a wavelength of 280 nm, the 260/280 ratio was used as the purity indicator of the DNA specimens. Since an optimum value for 260/280 ratio for pure DNA is 1.8, the percentage of specimens for each group with a purity ratio between 1.6 and 2.0 (1.8 ± 0.2) was additionally measured. DNA purity was finally determined according to 260/280 ratio as follows; 260/280 ratio between 1.8 and 2.0 was accepted as pure for DNA, and less than 1.8 or 2.0 or more was judged as bad. A ratio lower than 1.8 was considered as indicative of the presence of protein, phenol, or other contaminants that absorb strongly at or near 280 nm. And ratio of more than 2.0 was assumed as indicative of RNA

contamination.[12]

DNA integrity analysis by electrophoresis

To observe DNA integrity, 50 ng of each DNA specimen based on spectrophotometric measurement was analyzed by electrophoresis on a 2% agarose gel (Biosesang, Korea). Lambda DNA (Bioneer, Korea) and 1 Kb DNA ladder (Bioneer, Korea) were used as positive control and DNA molecular weight marker, respectively. After loading and running for 2 hours at a voltage of 50 V, the results were analyzed using a UV transilluminator. Well-preserved DNA appeared as a unique, well-defined high molecular weight band higher than 20 kb. DNA was determined to be degraded when smearing was observed.[13]

***KRAS* mutation status analysis by peptide nucleic acid (PNA) clamping**

To evaluate the feasibility of *KRAS* molecular testing in biobanked specimens, *KRAS* exon 2 (codon12 and 13) mutation analysis by PNA-mediated real-time PCR clamping was performed on biobanked frozen tissue in all specimens. The amount of DNA used for PNA clamping was 40-80 ng/test (10 ng/reaction). A PNAClamp™ KRAS Mutation Detection Kit (Panagene, Daejeon, Korea) was used to detect KRAS mutations by real-time PCR, according to the manufacturer's instruction. Briefly, all reactions included template DNA, a primer and PNA probe set, and the SYBR Green PCR master mix. Real-time PCR was performed using a CFX96 PCR detection system (Bio-Rad, Philadelphia, PA, USA). The efficiency of PCR clamping was determined by measuring the threshold cycle (Ct) values. Higher ΔCt ($\Delta Ct = Ct_{standard} - Ct_{specimen}$) values meant that the mutant DNA was efficiently amplified. A cutoff value of 2.0 was used to indicate the presence of mutant DNA. *KRAS* mutation status of all 45 biobanked frozen tissues that was performed at this time was compared with the results of corresponding FFPE tissues that was initially performed at the time of pathologic diagnosis.

RESULTS

DNA purity of different CIT and SP

All DNA specimens had a 260/280 ratio ranging between 1.8 and 2.0 with the exception of a single specimen. The specimen with a ratio out of range belonged to group 5 (CIT-2/SP-2) and showed 1.76 of 260/280 ratio, suggesting the presence of protein or other contaminants that absorb strongly at or near 280 nm. DNA remained stable in CRC tissues kept at room temperature for up to 60 minutes (mean 260/280 purity ratio: 1.89 in CIT-1, 1.83 in CIT-2, and 1.90 in CIT-3) and long-term storage for up to 5 years (mean 260/280 ratio: 1.88 in SP-1, 1.87 in SP-2, and 1.87 in SP-3) (Table 1) (Fig. 1).

DNA integrity of different CIT and SP

All DNA specimens appeared as a compact, high-molecular-weight band with no or scanty low-molecular-weight smears in the gel electrophoresis. According to our results, 100% of extracted DNA specimens were of good quality

Table 1. Mean 260/280 ratio for DNA purity analysis in nine groups assigned by combination of cold ischemia time and storage period

Group	Mean 260/280 ratio
1 (CIT-1/SP-1)	1.91
2 (CIT-2/SP-1)	1.92
3 (CIT-3/SP-1)	1.86
4 (CIT-1/SP-2)	1.87
5 (CIT-2/SP-2)	1.85
6 (CIT-3/SP-2)	1.89
7 (CIT-1/SP-3)	1.91
8 (CIT-2/SP-3)	1.89
9 (CIT-3/SP-3)	1.93

CIT = cold ischemia time; SP = storage period; CIT-1 = less than 30 minutes; CIT-2 = 31-45 minutes; CIT-3 = 46-60 minutes; SP-1 = less than 1 year; SP-2 = 2-3 years; SP-3 = 4-5 years.

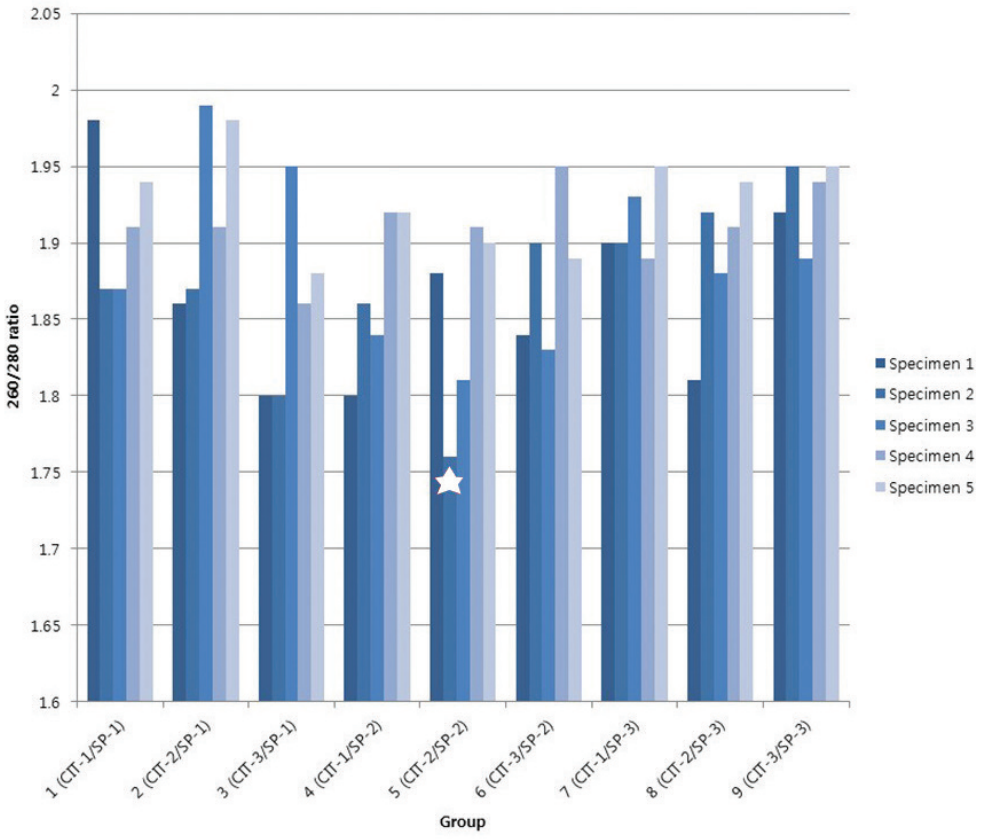

Fig. 1. DNA purity (260/280 ratio) in 45 biobanked fresh frozen colorectal cancer tissues. A single specimen showed out of DNA purity range with 1.76 of 260/280 ratio (★).
CIT = cold ischemia time; SP = storage period; CIT-1 = less than 30 minutes; CIT-2 = 31-45 minutes; CIT-3 = 46-60 minutes; SP-1 = less than 1 year; SP-2 = 2-3 years; SP-3 = 4-5 years.

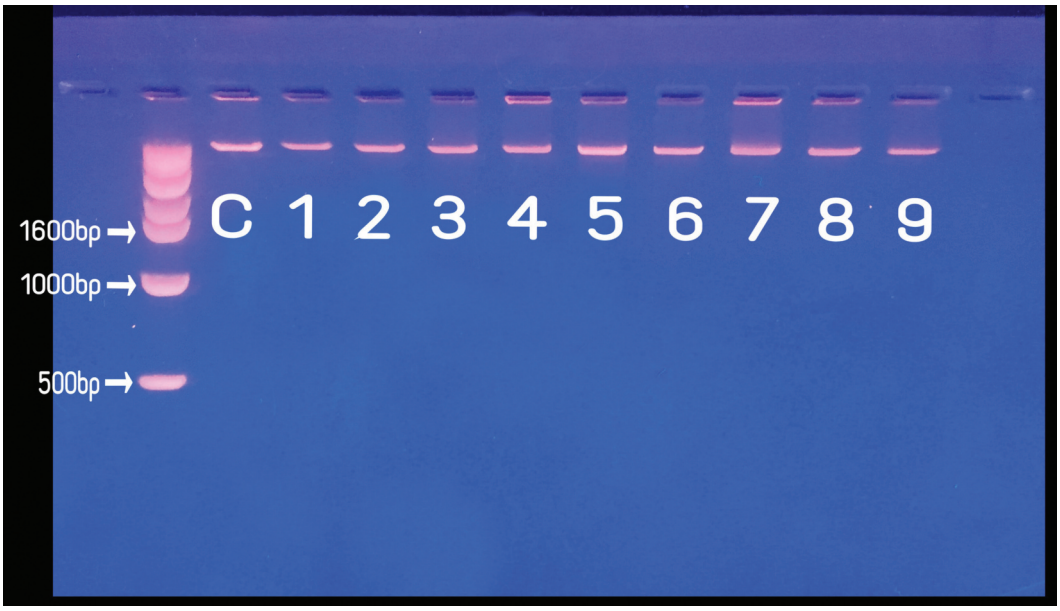

Fig. 2. Representative DNA integrity of nine groups confirmed by an UV transilluminator following electrophoresis with 2% agarose gel. Clear high molecular bands were observed in all biobanked specimens. C = control; 1-9 = group number.

and no significant difference was found in DNA quality of different CIT. DNA band from agarose gel results for group 1, 2, and 3 representing less than 1 year of SP appeared to be clearer than groups 4-9 representing more than 1 year of SP, but which showed no significant difference (Fig. 2).

KARS mutation status

The DNA quality was also assessed by PCR amplification of *KRAS* gene to evaluate practical usability of DNA from a biobanked tissue for molecular study. *KRAS* mutation status of all 45 biobanked frozen tissues that was performed at the time of current study was compared with the results of corresponding FFPE tissues that was initially performed at the time of pathologic diagnosis. The concordance of *KRAS* mutation status between all 45 paired biobanked frozen tissues and FFPE tissues was 100%, with 14 paired specimens showing the same *KRAS* mutations (Fig. 3). *KRAS* mutation was found in 31.1% (14/45) of CRC. The most common mutation locations were in codon 12 (64.3%, 9/14) and codon 13 (28.6%, 4/14). One specimen (7.1%, 1/14) had 2 synchronous mutations in codon 12 and codon 13. According to the PCR results, all the Ct values were stable at different CIT or SP.

DISCUSSION

Biobanking involves the collection, processing, transport, storage, and retrieval of biospecimens for future researches.[2] Biobanked specimens are considered as invaluable future resources to carry out molecular biology, cell biology, genetics, transcriptomics, genomics and proteomics re-

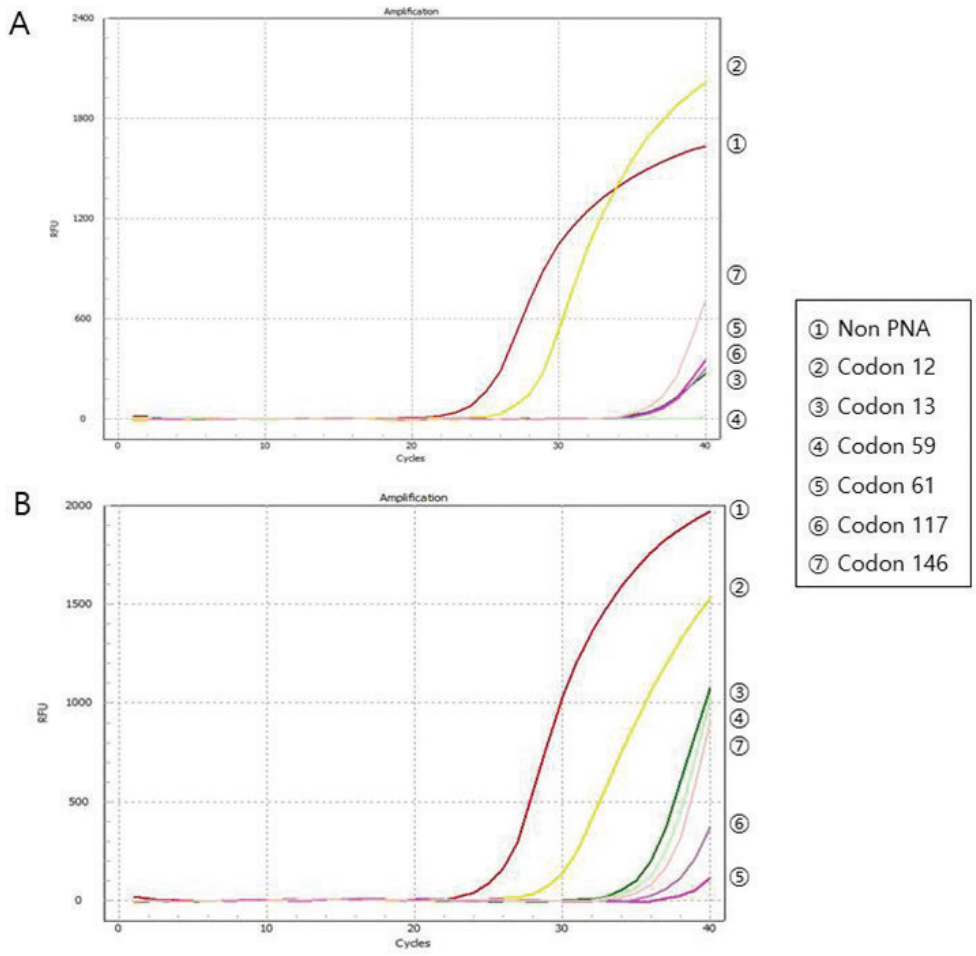

Fig. 3. Representative *KRAS* mutation status. A colorecatal cancer in group 9 (cold ischemia time 46-60 minutes and storage period 4-5 years) showed identical *KRAS* codon 12 mutation in both biobanked fresh frozen tissue [A] and formalin-fixed paraffin-embedded tissue [B].

search in order to explore new standards of tumor classification, diagnosis, treatment, and prognosis.[8]

However, the quality of DNA or RNA expression in biobanked specimens is dependent on multiple preanalytical variables such as tissue type, intrinsic patient factors, warm IT (i.e., extraction of the specimen after ligation of the large vessels), CIT, fixation method, subsequent transport, storage condition, and SP of specimens.[14] While tissue type and intrinsic patient factors cannot be modified, other factors such as CIT and SP can be controlled. Although several studies reported the impact of CIT and SP on DNA integrity of variable biobanked tissue, it is not well documented.[5-10,15] For CRC tissue, one study reported that a critical time point for tissue handling appeared to be 1 hour at room temperature,[15] whereas other studies have shown that RNA remained stable when kept at room temperature and on ice for up to 4 hours.[16] A recent review specifically addressing the effect of CIT on RNA

stability concluded that in most of the studies, only minimal changes (≤ 10%) in the RNA integrity number in CRC tissues were observed during a CIT of 1 – 6 hours.[9] Guerrera et al.[17] reported that patient-derived xenografts engraftment rate was adversely affected by prolonged CITs (> 10 hours). Regarding SP, extended cryogenic storage beyond 2-11 years remained a viable option for maintaining the high quality of various tissue specimens in biobanks.[10] Long-term storage of biobanked CRC specimens did not negatively influence DNA and RNA qualities, for 2 years[14] and up to 40 months.[16] In the present study, we found no significant difference in DNA purity and integrity of different CITs (up to 1 hour) and SPs (at least 5 years) similar to other studies. Apparently, it is evident that storage conditions of our biobank are suitable for long-term specimen preservation with high DNA quality.

To determine the suitability of biobanked frozen tissue for biomarker research according to various CITs or SPs, the mutation status of *KRAS* was investigated in all paired FFPE and biobanked frozen tissues. *KRAS*, a well-known signaling molecule in the EGFR pathway, has been recognized as one of the most frequently mutated oncogenes in CRC.[11] Mutations in codons 12/13 of *KRAS* exon 2 are associated with reduced benefit from anti-EGFR antibody treatment for metastatic CRC.[18] Our study showed 100% concordance of *KRAS* mutation in all 45 paired FFPE and biobanked frozen tissues with variable CITs and SPs, thereby suggesting that biobanked tissues with 1-hour CIT and 5 years SP are suitable for biomarker research. In the near future, significant advancements in tissue-sequencing platforms will be made to understand molecular characterization and discover novel biomarkers for predicting treatment response for CRC patients. Consequently, there is a need to reconsider the utility of specimens stored beyond a certain timeframe. In this context, high-quality biobanked frozen tissue would hold practical significance with utilization as alternative biospecimens to investigate future relevant molecular signatures, when archival FFPE block is not available.

Despite the advantages of FFPE tissues including abundance and availability, connection to rich clinical data, and association with patient outcomes,[19] another issue involves C > T transition artifacts associated with FFPE-derived DNA as a result of the addition of adenine instead of guanosine due to deamination.[20] Previous studies demonstrated that pairwise analysis of DNA exome-sequencing data showed concordance for 70 - 80% of variants in biobanked frozen tissue and FFPE specimens stored for fewer than 3 years[21] and next-generation sequencing results from biobanked frozen specimen were more consistent than FFPE specimens.[22] Although RNA-sequencing data showed a high correlation between expression profiles in biobanked frozen tissue/FFPE irrespective of storage time (up to 244 months) and tissue type, there exist discordances between RNA-sequencing results from FFPE and biobanked frozen tissue.[21] In addition, high failure rate in FFPE specimens to achieve sufficient quality RNA for RNA-sequencing has

been demonstrated.[19] FFPE tissue processing and specimen storage are known to result in highly degraded and chemically modified RNA, which limits gene detection and introduces sequencing artifacts.[19,23] Unlike FFPE tissue, the DNA and RNA from frozen biospecimens are generally of high molecular weight and without cross-linking, which are ideal for a wide variety of purposes such as whole-genome amplification, whole genome sequencing, and cDNA microarray analyses, when the tissue biobanking is precisely controlled under the SOP.

In this context, all tissue specimens for biobanking must be precisely handled under the SOP. SOP addresses multiple factors including collection, preparation, transport, storage, management, instrumentation, facilities, quality control, and utilization of fresh tissue for standardized specimen consistency, accuracy, and quality.[24] The SOP is also needed to avoid high costs and the disposal of valuable biospecimens. Furthermore, researchers carry out large-scale, multicenter studies and SOP is required to standardize different collections and for quality management of high-quality specimens within different institutions to assure good scientific practice.[22] Since the Korea Biobank Project started in 2008,[25] many medical college- or hospital-based biobanks have been in operation in Korea. As the SOP for the biobank of Korea was developed quite recently,[25,26] there is lot of unexploited potential for scientific questions. We hypothesize that this study would partly contribute towards standardization of collection, storage, and quality control of fresh frozen tissues in Korea biobank.

There are a few limitations to our study. Firstly, this study did not represent the full diversity of the preanalytical tissue handling process in tissue biobanking and could not perfectly exclude other factors having a major impact on DNA quality. However, we were able to analyze the most important modifiable factors such as CIT and SP and show actual specimen handling data as per institutional review board-approved SOP. Another limitation is that the present study only provided the influence of CIT and SP on DNA quality. As ischemia has a more significant influence on RNA integrity, results should thus be confirmed based on analysis of RNA according to preanalytical variables for performing emerging systematic techniques such as next-generation RNA sequencing. The third limitation is that the present study could not provide data on cold ischemia tolerance according to specific tissue types, because the subject studied was only CRC tissue. Further studies involving various tissues are necessary to elucidate whether other tumors demonstrate similar quality results and feasibility of collection protocols for specific tissue types. Finally, our biobank has been established only for a short period (presently up to six years), further studies with increased CIT and SP points are necessitated to validate this observation and find optimal CIT and SP.

In conclusion, we found no significant difference in DNA purity and integrity of different CITs (up to 1 hour) and SPs (at least 5 years) in biobanked CRC specimens. Moreover, the storage conditions of our biobank are considered suitable for biomarker research involving high DNA quality.

The findings of this study have practical implications that could affect biospecimen collection and banking guidelines.

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Clinical Usefulness of Contrast-Enhanced Computed Tomography in Patients with Non-Obstructive Acute Pyleonephritis

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Objectives: The aim of this study is to investigate the clinical utility of contrast-enhanced computed tomography (CE-CT) in patient with non-obstrcutive acute pyelonephritis (APN).
Methods: From 2007 to 2013, 537 APN patients who underwent a CE-CT scan within 24 hours after hospital admission were enrolled. We divided these patients into greater (50% or greater involvment, n = 143) and lesser (less than 50% involvement, n = 394) groups based on renal parenchymal involvement in CE-CT examination. We compared clinical characteristics between two groups and analyzed the clinical value of CE-CT scan as a reliable marker for predicting clinical severity and disease course in patient with non-obstructive APN.
Results: The mean estimated glomerular filtration rate was 70.6 ± 25.5 mL/min/1.73m^2. Compared with patients in lesser group, the patients in greater group had lower serum albumin levels (3.5 ± 0.5 vs 3.8 ± 0.6, $P < 0.01$) and longer hosptal stay (10.1 ± 4.7 vs 8.8 ± 4.5, $P < 0.05$). In addition, acute kidney injury (AKI) (23.1% vs 11.4%, $P < 0.005$) and bacteremia (36.4% vs 26.8%, $P = 0.02$) were frequently developed in greater group, respectively. The overall incidence of AKI was 14.8% based on RIFLE criteria. In a multivariate logistic regression analysis for predciting AKI, age, presence of diabetes mellitus and the presence of renal parenchymal involvement of greater than 50% in CE-CT were significant predictors of AKI.
Conclusions: The CE-CT scan could be useful to predict the clinical severity and course in non-obstructive APN patients with preserved renal function.

Key Words: Acute kidney injury, Computed tomography, Pyelonephritis

Acute pyelonephritis (APN) is defined as an acute inflammation of the upper urinary tract, including renal parenchyma, calyces, and pelvis. APN usually presents as mild diseases, but it sometimes causes substantial morbidity and mortality.[1] Several parameters including old age, immunosuppression, health care associated infection, obstructive uropathy, decreases in platelet count, serum albumin level, high C-reative protein, and bacteremia have been proposed as prognostic factors predicting poor prognosis in patients with APN.[2-4]

Contrast-enhanced computed tomography (CE-CT) is used for diagnosis of APN, and can provide important information on the range of inflammation and other accompanying compli-

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cations to help to establish a precise diagnosis.[5,6] There are some specific CT findings observed in APN patients. The most common radiologic CE-CT finding of APN is a striated or wedge-shaped area of hypoperfusion after contrast injection.[7,8] However, there have been few studies about correation between the extent of infection in renal parenchyma and non-obstructive APN severity.[7,8] In the present study, we evaluated the clinical utility of CE-CT for predicting clinical severity in patient with non-obstrcutive APN.

METRIALS AND METHODS

Patient selection

From January 2007 to December 2013, we enrolled 556 APN patients who underwent a CE-CT scan within 24 hours after hospital admission before starting antibiotic treatment. Patients were excluded if they were younger than 18 years or had an hydronephrosis or renal abscess on CT scan. We also excluded the patients with post-contrast induced-acute kidney injury (CI-AKI) or low-quality image due to various causes. A clinical diagnosis of APN was made in patients with more than three of the following five diagnostic criteria: (1) clinical symptoms such as fever, chills, vomiting, or flank pain (2) costovertebral angle tenderness (3) fever of higher than 37.5 ℃ (4) leukocytosis in the complete blood count (5) abnormal urine test results (pyuria: white blood cell of ≥ 10/high-power field or positive urine culture of 10^5 colony-forming unit/mL). A total of 537 patients were included in this study. Two experienced radiologist divided these patients into two groups by approximately quantifying renal parenchymal low density extent on CE-CT. APN extent was approximately quantified on CE-CT by calculating number of slices that contained less enhancing lesion divided by total number of slices that contained normal renal parenchyma (Fig. 1). We also checked the status of renal parenchymal involvement. In cases with bilateral APN of different grades in each kidney, the higher grade was recorded. Patients were divded into two groups: greater group (greater than 50% involvement, n = 143) and lesser group (lesser than 50% involvement, n = 394). This study was approved by the Institutional Review Board of the Presbyterian Medical Center, Jeonju, South Korea (IRB No. 2019-05-014).

Clinical and laboratory information

All patients had a detailed clinical history and examination, a standard set of investigations including complete blood counts, liver function tests, serum creatinine, urea, electrolytes, chest radiograph, three peripheral blood smears for malaria, urinalysis, and two blood cultures. AKI was defined based on the RIFLE (Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease) criteria,[9] and patients were categorized into the R, I or F categories. We defined CI-AKI with the RIFLE criteria as a relative increment in serum creatinine of ≥ 50%, or a decrease in estimated glomerular filtration rate of ≤ 25% from baseline, or an episode of oliguria lasting ≥ 6 hours within 48 to 72 hours following

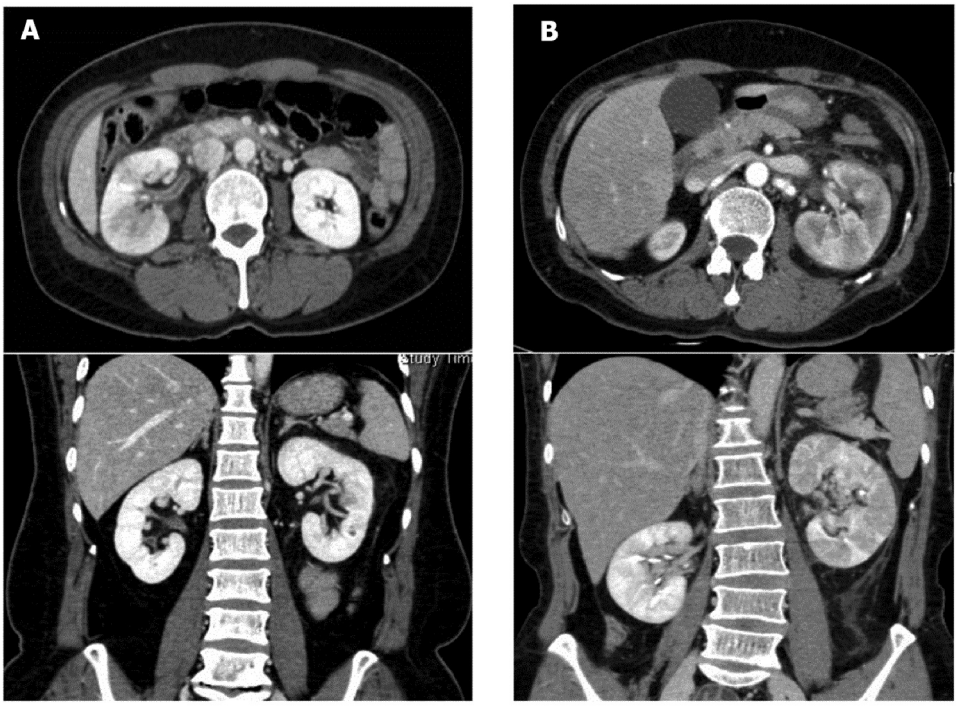

Fig. 1. Acute pyelonephritis grades according to CT findings. (A) lesser grade, lesser than 50% of renal involvement (B) greater grade, greater than 50% of renal involvement.

contrast administration.[10] The estimated glomerular filtration rate (eGFR) was calculated using the abbreviated Modification of Diet in Renal Disease (MDRD) equation.[11] When the baseline serum creatinine was not available, it was calculated using the standard four-variable MDRD formula assuming eGFR of 75 mL/min/1.73 m^2. The RIFLE class was determined based on the worst among serum creatinine levels, eGFR, and urine output criteria. Renal replacement therapy was initiated using the standard indications. Sepsis is defined as the presence (probable or documented) of infection together with systemic manifestations of renal infection. The CT images were reviewed by two radiologists who unaware of clinical information; the final decision of grade was reached by consensus.

CT technique

CT scan was conducted on a 16-channel multidetector row scanner (Somatom Sensation 16, Siemens Medical Solutions, Erlangen, Germany) with setting of 3 mm slice thickness. The CT findings were evaluated in the neprographic phases. Intravenous contrast material was administrated in an antecubital vein with injector at a dose of 1.5 mL/Kg body weight at a rate of 3 mL/s to a maximum of 120 mL. Nephrographic-phase scans were started 90 to 100 seconds after contrast injection.

Statistical analysis

All data are presented as means ± standard deviations unless otherwise specified. The baseline characteristics of patients in the greater and

Table 1. The clinical and laboratory findings of the 537 patients with nonobstructive acute pyelonephritis

Characteristics	
Age, years	55.2 ± 17.9
Female, n (%)	504 (93.9)
Co-morbidity	
Diabetes, n (%)	82 (15.3)
Hypertension, n (%)	157 (29.2)
Duration of hospital stay, days	9.2 ± 4.6
Sepsis, n (%)	7 (1.3)
Serum creatinine, (mg/dl)	0.9 ± 0.3
eGFR adm, ml/min/1.73m^2	70.6 ± 25.5
Total WBC count, (× 10^3/ mL)	12.7 ± 12.3
Serum albumin, (mg/dl)	3.8 ± 0.6
Bilateral involvement, n (%)	98 (18.2)
Bacteremia, n (%)	158 (29.4)
AKI, n (%)	78 (14.5)

eGFR adm: eGFR at the time of admission
WBC: white blood cell
AKI: acute kidney injury

lesser groups were compared using t tests for continuous variables and chi-square tests for categorical variables. Clinically relevant parameters or the variables that were significantly associated with the presence of AKI in the univariate analysis were included in the multivariate analysis. A *P*-value of < 0.05 was considered to be statistically significant. All statistical analyses were carried out using SPSS version 22.0.

RESULTS

Baseline characteristics

The baseline characteristics of the 537 study subjects are presented in Table 1. The patients included 504 (93.9%) men and 33 (6.1%) women, with a mean age of 55.2 ± 17.9 years. The mean estimated glomerular filtration rate was 70.6 ± 25.5 mL/min/1.73m^2. The initial white blood cell count (WBC) and serum albumin levels were 12.7 × 10^3/mL and 3.8 g/dL, respectively. The mean hospital stay was 9.2 ± 4.6 days. Bilateral renal involvement in CE-CT and bacteremia were noted in 18.2% and 29.4% of the patients, respectively. Of 537 participants, 78 (14.5%) experienced AKI during hospitalization period.

Comparison of clinical characteristics between greater and lesser group

When we compared clinical characteristics between greater (n = 143) and lesser (n = 394) groups, the patients in greater group had lower serum albumin levels (3.5 ± 0.5 vs 3.8 ± 0.6, P < 0.01) and longer hospital stay (10.1 ± 4.7 vs 8.8 ± 4.5, P < 0.05). Furthermore, bilateral renal in-

Table 2. Comparison of baseline characteristics between non-AKI and AKI group

	Greater (n = 143)	Lesser (n = 394)	*P*-value
Age	57.5 ± 16.6	54.4 ± 18.3	NS
Female, n (%)	137 (95.8)	367 (93.1)	NS
Comorbidty			
Diabetes, n (%)	26 (18.2)	56 (14.2)	NS
Hypertension, n (%)	50 (35.0)	107 (27.2)	0.05
Duration of hospital stay, days	10.1 ± 4.7	8.8 ± 4.5	< 0.05
Sepsis, n (%)	1 (0.70)	6 (1.51)	NS
eGFR adm, ml/min/1.73m^2	63.8 ± 23.3	72.8 ± 25.8	< 0.05
eGFR dis, ml/min/1.73m^2	71.4 ± 20.5	76.5 ± 23.5	NS
Total WBC count, (× 10^3/ mL)	13.8 ± 12.8	12.3 ± 12.0	NS
Serum albumin, (mg/dl)	3.5 ± 0.5	3.8 ± 0.6	< 0.05
Bilateral involvement, n (%)	40 (10.2)	58 (40.6)	< 0.05
Bacteremia, n (%)	52 (36.4)	106 (26.9)	< 0.05
AKI, n (%)	33 (23.1)	45 (11.4)	< 0.05

eGFR $_{adm}$: eGFR at the time of admission
eGFR $_{dis}$: eGFR at the time of discharge
WBC: white blood cell
AKI: acute kidney injury

Table 3. Predictors of development of AKI (univaritive and multivariative analysis)

	Univariate HR (95% CI)	*P*-value	Multivariate HR (95% CI)	*P*-value
Age	1.037 (1.021 - 1.054)	< 0.001	1.024 (1.005 - 1.043)	0.014
Diabetes mellitus	2.835 (1.622 - 4.954)	< 0.001	2.005 (1.080 - 3.724)	0.028
Hypertension	3.277 (2.004 - 5.358)	< 0.001	1.592 (0.879 - 2.886)	0.125
Serum albumin, (mg/dl)	0.367 (0.232 - 0.583)	0.019	0.660 (0.387 - 1.132)	0.125
Total WBC count (× 10^3/ mL)	1.049 (1.012 - 1.087)	0.006	1.031 (0.997 - 1.067)	0.073
50% or greater renal parenchymal involvement in CE-CT	2.327 (1.414 - 3.827)	0.001	1.901 (1.092 - 3.309)	0.023

AKI: acute kidney injury
WBC: white blood cell
CE-CT: contrast enhanced computed tomography

volvement (40.6% vs 10.2%, $P < 0.05$) and bacteremia (36.4% vs 26.9%, $P < 0.05$) were frequently developed in greater group, respectively (Table 2). AKI (23.1% vs 11.4%, $P < 0.05$) occurred more frequently in greater group than lesser group. According to the RIFLE criteria, 52

(66.6%) and 26 (33.4%) in our study fell into the R and I categories, respectively. By univariate analysis, age, presence of diabetes mellitus or hypertension, serum albumin, total leukocyte count, bilateral renal involvement and 50% or greater renal parenchymal involvement in CT scan were significant predictors of AKI. Adjusting for these factors in a multivariate logistic regression analysis, older age and CT finding of 50% or greater renal parenchymal involvement were the only significant predictors of AKI (Table 3).

DISCUSSION

In the present study, we show that the patients in greater group had longer hospital stay than lesser group. In addition, compared with lesser group, AKI and bacteremia were frequently developed in greater group. Our findings provide a rationale for using CE-CT scan as a tool for predicting clinical severity in patients with non-obstructive APN.

APN is the most common form of upper urinary tract infection, and usually occurs secondary to an ascending infection of gram-negative bacteria in women.[12] Although APN is easily treated by antibiotics, its clinical course and severity varies.[1] Several risk factors are associated with the more severe presentations of urinary tract infection. Of these, urinary tract obstruction was independent predictor of septic shock.[3,13] To evaluate urinary tract obsctrution due to various causes, radiological tests including CT or ultrasound are needed. In case of non-obstructive APN, the most common radiologic CT finding is a striated or wedge-shaped area of hypoperfusion or mass-like lesion after contrast injection.[7,8] However, there are few data about such CT finding as a marker reflecting APN severity.[7,8,14]

Paick et al. graded APN into 4 groups according to the extent of renal involvement; no renal parenchymal involvement as grade 1; less than 25% involvement as grade 2; 25% to 50% involvement as grade 3; and greater than 50% as grade 4, and reported clinical usefullnes of CT grade for predicting clinical course of APN.[14] In presenting study, we classified the subjects into two groups based on modification of method reported by Paick et al. Initially, we also classified the subjects into four groups according to APN grade reported by Paick et al, however, there were no statistically signficant differences among groups (the data were not shown). This finding migth be due to the difference of enrolled participants. In the study by Paick et al, the mean age of the study population was 39.3 years and there were no patients with AKI. On the other hand, the mean age in our study was 55.2 years, and the overall incidence of AKI was 14.5%. Thus, large prospective randomized controlled study is needed to check whether the assocation of the extent of renal involvment in CT and clinical severity can differ based on renal function. However, the increase in hospital stay of greater group, which is shown in our study, was also observed in the study reported by Paick et al.

Urothelial thickening, diffuse peritoneal thickening, perinephric fat infiltration and the presence of two or more abonormal CT findings were

more frequently observed in patients with bacteremic APN.[15] However, there are few data about correation between bacteremia and extent of renal involvement.[16] In case of patients with APN, blood cultures have been reported to be positive in 18-32% of APN cases.[16-18] In our study, 158 (29.4%) patients out of 537 have positive blood culture, and bacteremia (36.4% vs 26.9%, $P < 0.05$) were frequently developed in greater group. During treatment of APN, it is important to differentiate bacteremic urinary tract infection (UTI) patients from non-bacteremic UTI patients since complication of bacteremic UTI can lead to sepsis or death. Thus, our data show that CT finding may be helpful to predict bacteremia in patients with APN.

Old age, arteriosclerosis, diabetic vasculopathy, chronic hypertension, and chronic kidney disease are regarded as risk factors for renal failure due to impairment of the vasodilatory response in the afferent arteriole.[19,20] In our study population, old age and 50% or greater renal parenchymal involvement in CT examination were significant risk factors for predicting APN-associated AKI. In this study, all patients with AKI recovered without renal replacement therapy after appropriate antibiotics and supporive care. According to our data, CT finding could be helpful to predict AKI in APN patients with preserved renal function.

Our study had certain limitations. First, this was a retrospective single center study. Thus, a large, prospective, randomized, controlled multicenter study is needed in the future. Second, the subjects in present study had relativley preserved renal function since patients with poor renal function did not undergo CE-CT due to CI-AKI. Third, although several CT findings were observed in APN,[15] we only investigated renal parenchymal involvement in CT. Thus, a large study using various CT findings in APN patients is needed in the future.

In our study, the patients with 50% or greater renal parenchymal involvement in CE-CT had severe type of APN based on hospital stay, prsence of bacteremia and AKI. Therefore, CE-CT could be helpful to discriminate patients with severe from non-severe APN patients in non-obstructive patients with preserved renal function.

Declaration of interest

All authors have no conflicts of interest to declare.

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Dynamic Change of Ischemic Mitral Regurgitation in a Patient with Acute Coronary Syndrome

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Ischemic mitral regurgitation (IMR) is commonly known as a chronic complication of left ventricular remodeling due to coronary artery disease. Acute IMR after coronary artery disease, such as acute myocardial infarction particular, could also develop as a mechanical complication involving papillary muscle rupture. However, the clinical significance of acute transient IMR and the therapeutic intervention in coronary artery disease is infrequently reported. We describe a patient with acute pulmonary edema due to acute IMR, which resolved immediately after coronary revascularization.

Key Words: Acute coronary syndrome, Mitral valve regurgitation, Pulmonary edema

Ischemic mitral regurgitation (IMR) has been reported to have chronic, acute, and transient forms. Chronic IMR is generally known as a consequence of left ventricular remodeling and mitral leaflet and chordae tethering. Acute IMR is an uncommon but serious complication of myocardial infarction that is associated with posterior papillary muscle rupture and dysfunction, requiring surgical intervention.[1] Acute reversible IMR, although less frequently reported, occurs in patients with normal left ventricular size, systolic function, and mitral valve structure.[1,2] Further, only a few studies have investigated the importance and treatment of acute transient IMR. Here, we describe a 71-year-old man who had a history of unstable angina, which led to acute mitral regurgitation (MR). The MR was resolved immediately after percutaneous coronary intervention (PCI).

CASE

A 71-year-old man with chest pain, which started after exercise, was admitted to the emergency room. He had a history of diabetes mellitus and stable angina with mild left anterior descending artery (LAD) stenosis, which was diagnosed 10 years earlier. Physical examination revealed clear lung sounds without crackles. An

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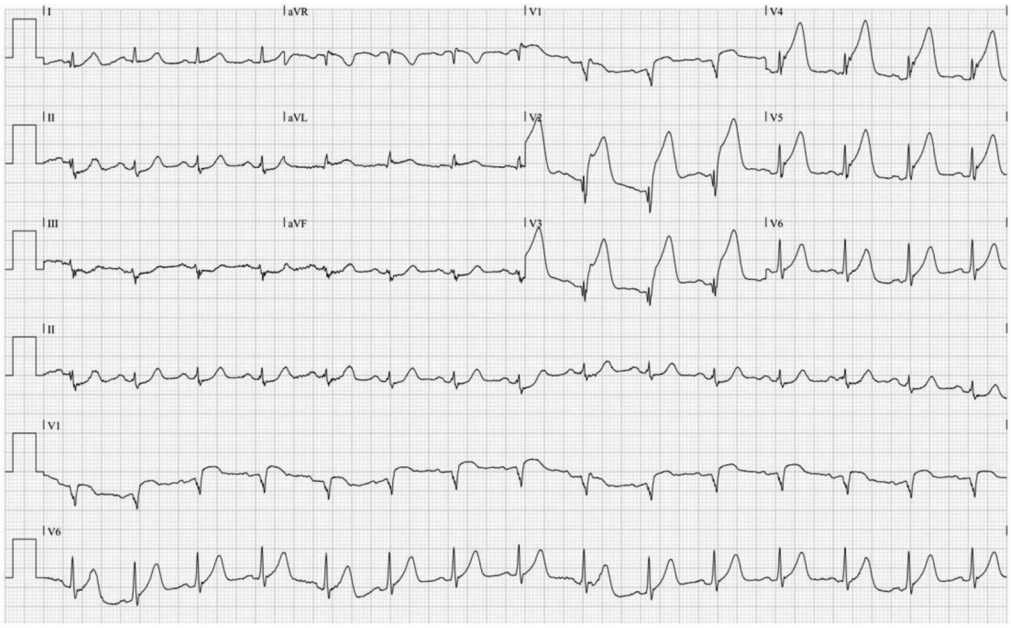

Fig. 1. Initial electrocardiogram shows extensive ST segment elevation on precordial leads.

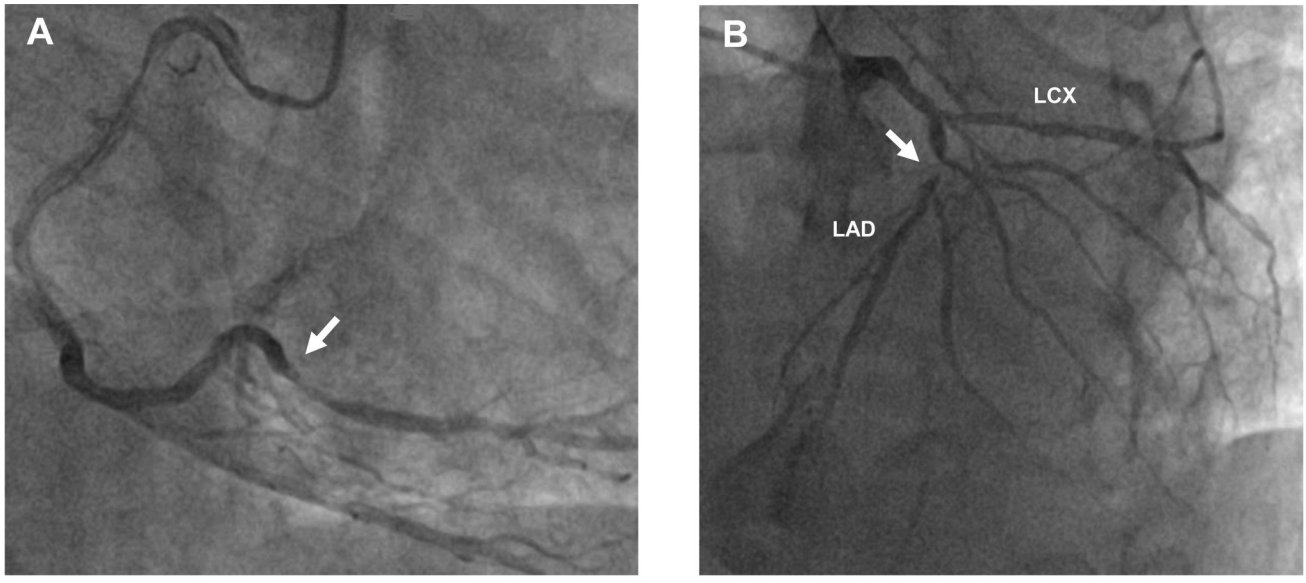

Fig. 2. Coronary angiography revealed about 70% stenosis of the distal right coronary artery (arrow, A) and near total occlusion on proximal left anterior descending artery (arrow, B). LAD, left anterior descending artery; LCX, left circumflex artery.

initial electrocardiogram showed ST segment elevation in the anterior leads (Fig. 1). Initial blood pressure was 110/70 mmHg, heart rate 74 beats/min, respiratory rate 18 breaths/min, O2 saturation 100% with room air. Primary PCI was performed, and it revealed near-total mid LAD occlusion and about 70% stenosis of the distal right coronary artery (RCA) (Fig. 2). For the cul-

prit coronary artery, mid LAD stenosis, thrombectomy and stent insertion (Synergy 2.5 × 24 mm, Boston Scientific Corporation, Marlborough, MA) were successfully performed. On transthoracic echocardiography (TTE) post-primary PCI, mild MR and hypokinetic motion of the mid to apical portion of the anterior wall were defined. Troponin I was elevated to > 23 ng/mL, creatine kinase-MB (CK-MB) was 300 ng/mL, and N-terminal pro B-type natriuretic peptide (NT-proBNP) level was 78.9 pg/mL. We decided not to perform any more intervention for the RCA and to start the medical therapy, including aspirin, ticagrelor, statin, a beta-blocker, and an angiotensin-receptor blocker. Ten days post-PCI, he presented with chest pain again, but he experienced relief after receiving a tablet of nitroglycerin sublingually. A repeat electrocardiogram showed normal sinus rhythm and no abnormal ST-T change, except the Q wave at the anterior precordial leads. There was no significant change in wall motion abnormality, left ventricular function, and valvular function on the follow-up echocardiography. Initial chest X-ray was clear without pulmonary edema. Treadmill exercise electrocardiography was performed according to the modified Bruce protocol. The test revealed ST segment elevation in aVR at stage 4 and ST segment depression in II, III, and aVF from stage 3 to the recovery phase. He complained of dyspnea immediately after the exercise. Troponin I and CK-MB levels were 0.13 ng/mL and 3.1 ng/mL, respectively. NT-proBNP level was elevated to 1,594 pg/mL. Chest radiography revealed diffuse bilateral infiltration, suggesting pulmonary edema. TTE demonstrated incomplete closure of the mitral valve with severe MR (Fig. 3). No additional wall motion abnormalities were noted; rather, mid-wall motion seemed to be improved. LV size and ejection fraction were within normal ranges. On performing coronary angiography, we confirmed that the previous LAD stent was patent and proceeded with RCA revascularization with stenting. TTE was repeated immediately after PCI and it revealed that the severe MR and incomplete coaptation were resolved (Fig. 3). The patient improved rapidly from a state of acute heart failure.

DISCUSSION

IMR is a frequent complication, and it also has important clinical impacts on the outcomes of ischemic heart disease.[2,3] However, its acute transient form has been reported infrequently. Moreover, using a left ventricular angiogram, Finelli et al.[4] demonstrated severe transient IMR in a patient with symptoms of recurrent heart failure. After introducing coronary revascularization technique, a few cases of resolution of acute IMR after coronary angioplasty have been reported.[5,6] Although the pathogenesis of transient IMR is unclear, posteromedial papillary muscle dysfunction due to ischemia is suggested to be the main causative mechanism. Acute severe IMR in acute myocardial infarction is known as an irreversible mechanical complication caused by papillary muscle rupture and/or dysfunction, which is related to pap-

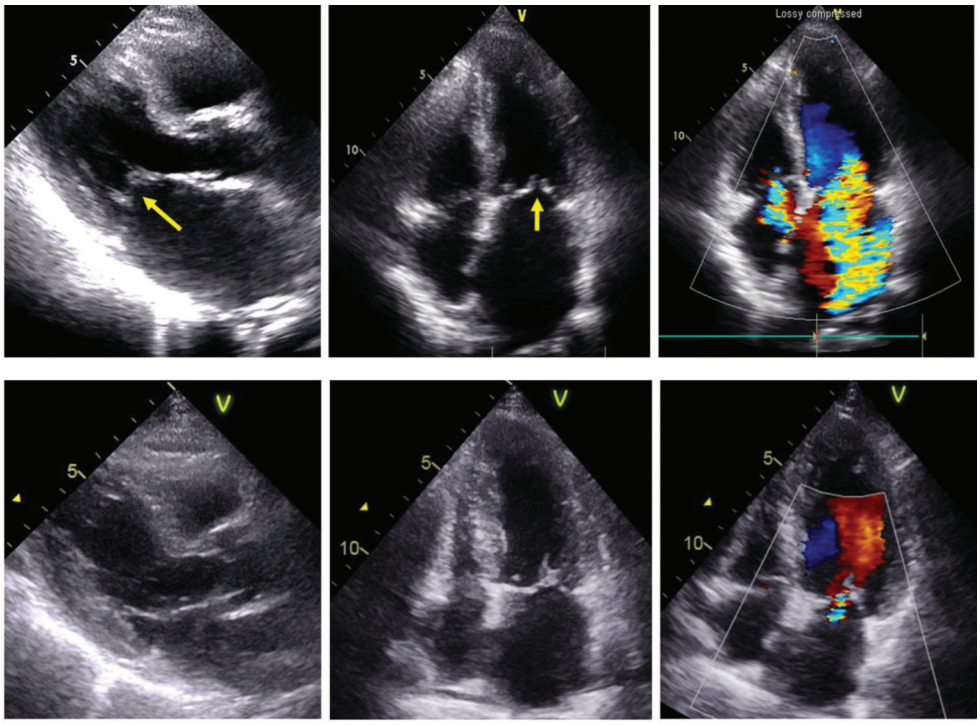

Fig. 3. Transthoracic echocardiograms show dynamic change of mitral regurgitation. Mitral leaflets do not coaptation on end-systole (arrows at parasternal long axis view and apical four chamber view images on upper panel) and color Doppler image shows severe mitral regurgitation (right, upper panel). On follow-up echocardiograms immediately performed after right coronary artery intervention (lower panel), mitral valvular coaptation and severe mitral regurgitation are completely improved.

illary muscle infarction. However, previous reports indicated that there is a transient form of IMR and that this IMR type could disappear after coronary angioplasty.[5,6] In our case, the patient with unstable angina who had minimal MR, acute severe MR and pulmonary edema were suddenly developed after treadmill exercise test. Remarkably, the acute severe IMR disappeared immediately after PCI for the RCA. To our knowledge, there is no echocardiographic documentation of abrupt IMR change in a patient with acute coronary syndrome. Recently, transient IMR due to coronary artery spasm was reported and the authors named it the "Eclipsed MR".[1,7,8] Liang et al. suggested that the mechanism behind transient IMR due to spasms is global subendocardial ischemia, which results in apical displacement of both papillary muscles.[1] They also explained that the ejection fraction appeared to be preserved because of an acute decrease in afterload caused by severe acute MR. The feature of TTE in our patient was similar to that in global subendocardial ischemia, although the main cause, which is localized posteromedial papillary muscle dysfunction related to RCA stenosis, is different. Therefore, coronary artery disease should be considered in the differential diagnosis of patients with transient IMR even in situations where the mitral valve is structurally normal,

wall motion abnormality is uncertain, and ejection fraction is preserved.The treatment of IMR remains inconclusive. In a population of patients with acute myocardial infarction, successful primary PCI was helpful in the improvement of IMR.[2,3] In the current case, significant RCA stenosis was recorded in the coronary angiogram for acute anterior ST-elevated myocardial infarction. During the second episode of unstable angina, severe IMR was detected. Because we considered the possibility of papillary muscle dysfunction due to RCA stenosis, early revascularization could be decided. In conclusion, acute transient IMR is an infrequently reported and under-recognized complication. We described a case of acute transient IMR caused by acute coronary syndrome, in which the patient fully recovered immediately after PCI. We suggest that coronary artery disease should be considered in the differential diagnosis of patients with transient IMR. Early diagnosis and prompt therapeutic intervention may help improve the prognosis of these patients.

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A Case of unexpected Fatal Hemoperitoneum in Non-severe Acute Pancreatitis

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Acute pancreatitis (AP) severity is determined by associated organ failure (OF). However, enzymatic erosion of peripancreatic vessels can lead to life-threatening hemoperitoneum in clinically non-severe AP without OF. We herein report a case of unexpected hemoperitoneum which developed in a patient with clinically resolving AP without OF. A 36-year-old woman with alcohol use disorder presented with resolving epigastric pain and sustained abdominal distension of 2 weeks' duration. Ranson's score on admission was 1 and Computed tomography (CT) revealed non-necrotic AP with peripancreatic fluid collection. She showed sudden hypotension with an abrupt decrease in serum hemoglobin within 24 hours after admission. She was suspected to have an acute hemoperitoneum associated with venous bleeding from AP based on repeated CT. Venous bleeding from the splenic branch was ligated during surgery. The possibility of bleeding at the pancreatic bed should be considered even if the pancreatitis is not severe.

Key Words: Alcoholic, Hemoperitoneum, Pancreatitis, Surgery

Anatomic complications develop only in 20% of acute pancreatitis (AP) cases.[1] The overall mortality associated with AP is less than 5%.[1] AP severity is determined by associated organ failure (OF).[2] However, enzymatic erosion of peripancreatic vessels can lead to life-threatening hemoperitoneum in both clinically non-severe and resolving AP even without OF. We herein report a case of unexpected hemoperitoneum that developed in a patient with clinically resolving AP without OF and was successfully treated using surgical ligation.

CASE

A 36-year-old woman with alcohol use disorder presented at the outpatient clinic with improving epigastric pain and sustained abdominal distension of 2 weeks' duration. She previously had several episodes of acute epigastric pain radiating to the back, which persisted for several hours, during two months and improved. She only had abdominal distension during the first visit, which makes deep breathing uncomfortable. She was previously diagnosed with hyper-

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tension, dyslipidemia, and polycystic kidney disease. She was taking bisoprolol and fenofibrate. Her history showed alcohol consumption of 1 bottle per day, 4 times a week, for 10 years. Her vital signs were stable with blood pressure 138/97 mmHg, heart rate 73 beats/minute, respiration rate 18 beats/minute, and body temperature 36.6°C. She did not appear to be acutely ill upon physical examination. Conjunctiva were not pale and sclera were anicteric. There was periumbilical tenderness and shifting dullness without abdominal guarding. Laboratory results revealed hemoglobin level 11.8 g/dL (hematocrit 35.3%), white blood cells count (WBC) 6,260/uL, and platelet count 211,000/uL. Prothrombin time (international normalized ratio, INR) was 1.39. Serum total protein and albumin levels were 4.8 and 2.6 g/dL, respectively, and aspartate aminotransferase and alanine aminotransferase levels were 55 and 13 IU/L, respectively. Alkaline phosphatase and gamma glutamyl transferase levels were 58 and 62 U/L, respectively, and total bilirubin level was 0.7 mg/dL. Serum amylase and lipase levels were 216 (normal, 22 - 80) and 565 (normal, 0 - 67) U/L, respectively, which revealed that the recent AP seemed to be resolving. Serum lactic dehydrogenase (LDH) level was 523 U/L and triglyceride was 113 mg/dL. Chest and abdominal x-ray scans were normal. Liver dynamic computed tomography (CT) was used to evaluate the possibility of chronic liver disease or pancreatitis related with chronic alcohol use 6 hours after admission. The liver surface and contour were unremarkable. However, there were multiple small cystic lesions in the pancreas with peripancreatic fat infiltration and fluid collection loculated at the head portion (Fig. 1). Additionally, there was a moderate amount of ascites. Diagnostic paracentesis was performed with ultrasound assistance at the right lower quadrant area. The ascitic fluid had a bloody appearance and serum ascites albumin gradient was 0.7. In ascitic analysis, total protein level 3.2 g/dL, albumin level 1.9 g/dL, LDH level 314 U/L, glucose level 139 mg/dL, red blood cell count 1,400,000/uL, and WBC count 1850/uL (neutrophil 13% and monocyte 72%) were found. We did not suspect hemoperitoneum because CT scan did not reveal any blood at initial evaluation and her vital signs were stable. She complained of dizziness and nausea on the second day of admission, 20 hours after the first CT scan. Her heart rate increased to 125 beats/minute without hypotension, 29 hours after the initial CT scan. Laboratory findings revealed that hemoglobin dropped to 6.9 g/dL from 11.8 g/dL. Serum amylase and lipase were 94 and 222 U/L. Follow-up CT scan revealed a newly developed hemoperitoneum in the left upper quadrant, left paracolic gutter, and pelvic cavity without active contrast media extravasation. After re-evaluation of the initial CT scan, undetected active leakage of contrast media from the mesenteric vessel at the level of duodenojejunal junction in the venous phase was found (Fig. 2). General surgery physicians had been closely monitoring the vital signs and decided to delay the time point for the exploratory laparotomy until the activity of internal bleeding is confirmed on radiologic findings. On the third day

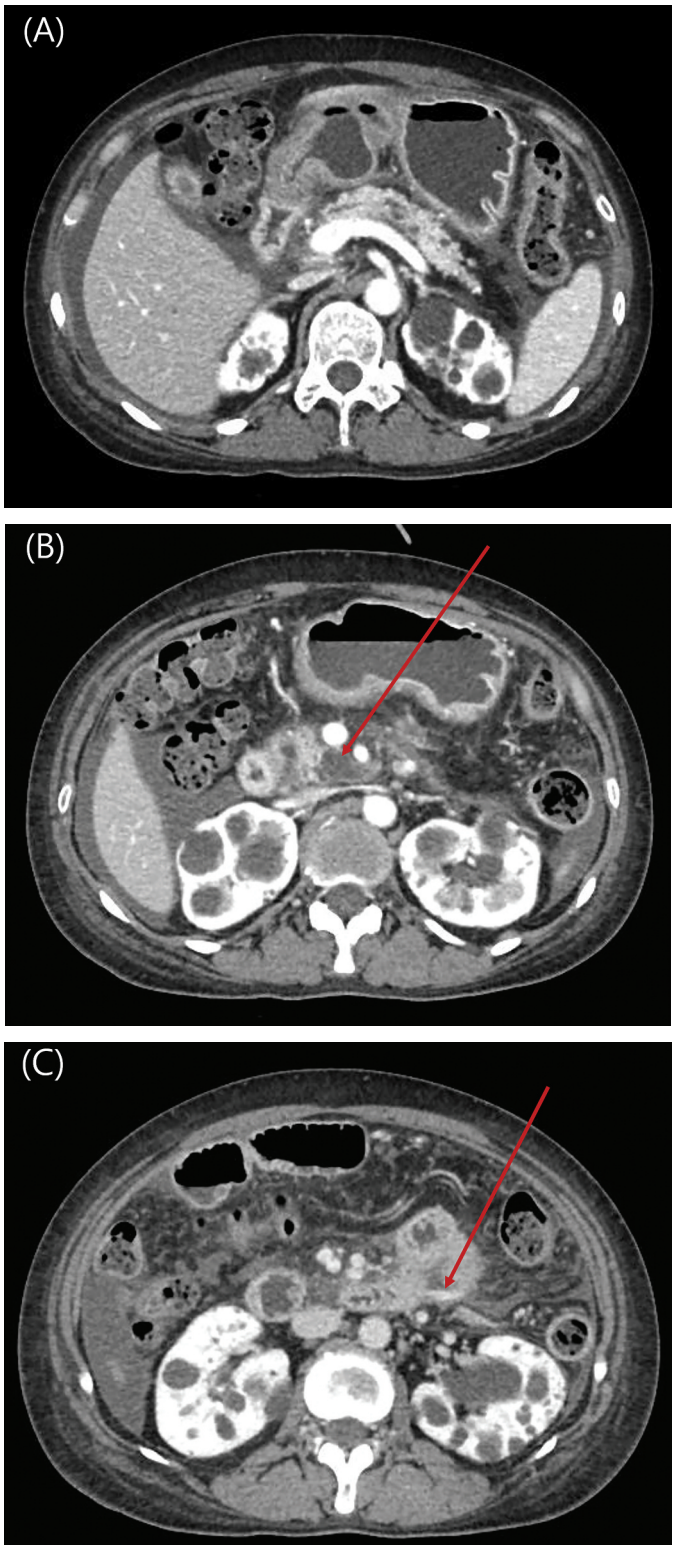

Fig. 1. Liver dynamic computed tomography (CT) scan of patient taken within 4 hours of admission.

(A) There are multiple cystic lesions in pancreas presumed to be small pseudocysts in portal phase.

(B) Peripancreatic fluid collection is notable at the pancreas head in portal phase (arrow).

(C) There is active leakage of contrast media from the mesenteric vessel at duodenojejunal junction in delayed phase (arrow).

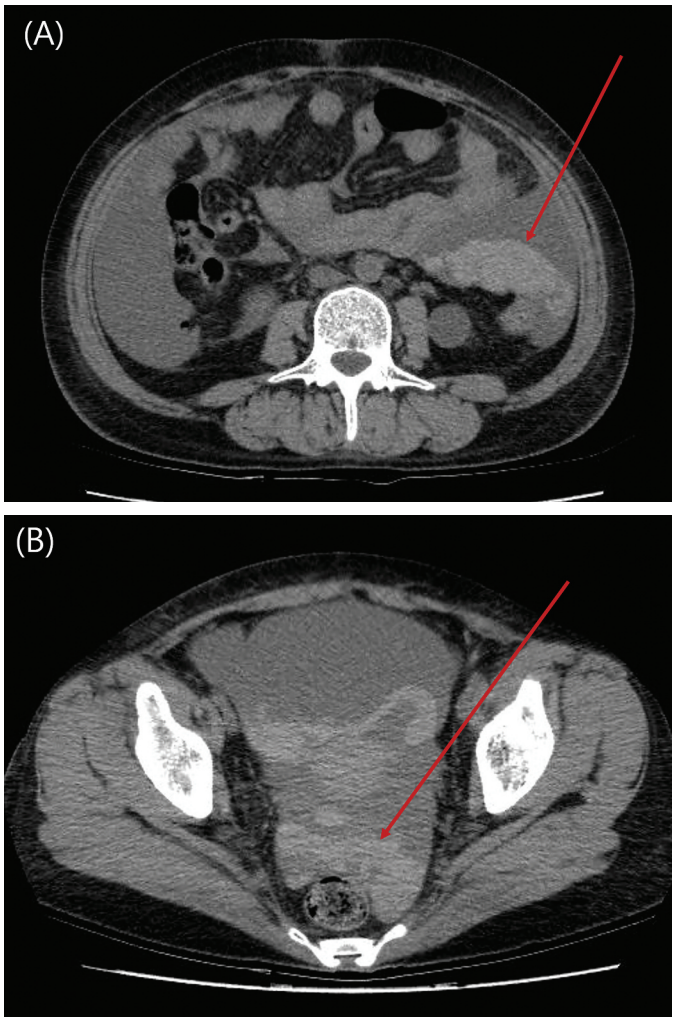

Fig. 2. Abdominal pelvis CT scan of patient taken after 29 hours of first CT scan immediately after clinical hypotensive event.

(A) There is evidence of hemoperitoneum in left upper quadrant in non-contrast enhanced image (arrow) without active leakage of contrast media in arterial phase.

(B) Newly developed hemoperitoneum is notable in pelvic cavity in non-contrast enhanced image (arrow).

and sixth day of admission, there were 2 events of hypotension (74/53 mmHg) and tachycardia (125 beats/minute) with less than 2 g/dL decrease of hemoglobin. Six units of packed red blood cells (PRBC) were used to compensate for continuous venous bleeding during 5 days of observation without significant collapse until laparoscopic evaluation. Finally on the seventh day, we conducted the surgery on the patient. 2 L of blood from the hematoma were evacuated in the operation room, but the focus of active bleeding was not found and the suspected focus visible on CT scan could not be ligated. Four more units of PRBC were transfused. After the first operation, about 1 L of fresh blood was consistently drained through hemovac during the first 24 hours with a single event of hypotension (76/51 mmHg) and tachycardia (121 beats/minute),

which led to a second emergency operation. During the operation, there was venous bleeding near the lateral border of the Treitz ligament from the splenic branch on the lower margin of the pancreas. The bleeding was controlled using surgical ligation. Further bleeding was not observed after the second operation and she was discharged 10 days later.

DISCUSSION

AP is an acute inflammatory process in the pancreas with or without the involvement of regional tissues or distant organs.[1] Disease severity is classified based on the presence of local/systemic complications and/or OF.[2] Without OF, severe AP is excluded by definition and in-hospital mortality of AP without OF is significantly lower than that of AP with any OF (2% vs. 28%).[3] Therefore, local complication itself is not the determinant in defining the severity.[2] However, acute intraabdominal hemorrhage, a rare type of local complication, is associated with high mortality rates similar to severe AP.[2,4]

We herein report a case of an unexpected hemoperitoneum which developed in a patient with clinically resolving AP without OF. At the time of diagnosis, epigastric pain which developed 2 months ago was improved and decreased amylase and elevated lipase levels revealed resolving pancreatitis. Although the CT scan revealed a Balthazar grade D AP without necrosis, Ranson's score was 1 on admission and 2 after 48 hours of admission (LDH level 523 U/L and 14% decrease of hematocrit). She could have been observed at an outpatient clinic because her symptoms were relieving. However, fatal hemoperitoneum developed within 24 hours of presentation and 10 units of PRBCs were transfused to sustain and stabilize her vital signs until the bleeding focus could be found and surgically ligated.

Acute bleeding into the gastrointestinal (GI) tract or intraabdominal cavity can develop from severe inflammation and liberated activated enzymes, which erode the local vessels during AP.[1,4] Ruptures of the splenic artery, splenic vein, and portal vein are frequently reported.[4] Other mechanisms include rupture of pseudoaneurysm of the splenic artery, pressure necrosis of vascular structure by pseudocysts, or pancreatic abscess.[1] Several large data pools exist regarding hemorrhagic complications in AP. However, it is confusing as the data also include GI bleeding, which was either pancreatitis- or non-pancreatitis related.[4-6] Recent data from a single-center study reported that the incidence of AP with hemorrhage into the pancreatic bed was 2.7%.[6] Mortality rate from intra-abdominal hemorrhage was reported to be 67%.[5] Diffuse peripancreatic bleeding occurred in both severe and mild forms of AP.[5]

We speculate that this is the first report of AP complicated by life-threatening hemoperitoneum without the presence of pancreatic necrosis or pseudoaneurysm. There was a case report of intra-abdominal bleeding from AP initially diagnosed with a Balthazar score of 2 and a Ranson's score of 2, which led to sudden cardiac death within 22 hours after admission.[7] The origin of

bleeding was not identified either through CT or angiography. In contrast to this, we were able to find the focus of bleeding using a timely taken CT scan, retrospectively. In addition, continuous intraperitoneal bleeding could successfully be ligated during an adequately timed surgery. There were several events of hypotension and tachycardia with marginal hemoglobin decreasing less than 2 g/dL, but it was difficult to decide when to proceed with surgery because those events were easily restored through intravenous hydration and PRBC transfusion. The first operation was conducted after 24 hours of tachycardia and the second operation was done immediately after the development of hypotension. To summarize, to reduce the failure of the surgery, it would be important to wait and see until the active bleeding occurs in the setting of pancreatic bed bleeding due to acute pancreatitis.

In conclusion, clinically non-severe (based on revised Atlanta classification), resolving AP can be complicated by a life-threatening hemoperitoneum. Even if there is no evidence of pancreatic necrosis or pseudonaeurysm of local vascular structures, the possibility of bleeding at the pancreatic bed should be kept in mind. Venous bleeding can be surgically treated at the same time as acute active bleeding.

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Atypical Lipomatous Tumor/Well-Differentiated Liposarcoma Arising from the Tongue: case report

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Liposarcomas are common mesenchymal malignant tumors arising from adipose tissue. Although liposarcomas are the most frequent type of soft tissue sarcomas, accounting for approximately 20% of all soft tissue sarcomas, they are rare in the head and neck, particularly in the oral cavity. Oral liposarcomas have been reported to occur mainly on the buccal mucosa, with other sites including the floor of the mouth, tongue, palate, and mandible. This report describes a 76-year-old male patient with an atypical lipomatous tumor/well-differentiated liposarcoma of the tongue that underwent surgical excision. This report also reviews published data on these rare tumors.

Key Words: Atypical lipomatous tumor, Tongue, Well-differentiated liposarcoma

Liposarcomas are mesenchymal tumors that occur in adipocytes, accounting for about 20% of adult soft tissue sarcoma. More than 60% of liposarcomas occur in the lower extremities and retroperitoneum, with 1.8 – 6.3% occurring in the head and neck area. Liposarcomas in the oral cavity are exceedingly rare, with few patients in Korea reported to have liposarcomas of the tongue.

Histopathologically, liposarcomas have several subtypes, including well-differentiated, dedifferentiated, myxoid/round-cell, pleomorphic and mixed-type liposarcomas. Most well differentiated liposarcomas in the oral cavity are predominantly superficial, are of small size, and have clear margins. Because these factors are associated with good patient prognosis, the World Health Organization (WHO) has classified well differentiated liposarcomas as atypical lipomatous tumors.[1]

To date, few patients in Korea have been reported with liposarcomas on the tongue. This report describes a rare atypical lipomatous tumor of the tongue. The tumor was successfully removed surgically, with no recurrence at 1-year follow up.

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CASE

A 76-year-old male patient presented with an asymptomatic, slowly growing tongue mass of unclear onset. The patient had undergone treatment for hypertension, diabetes, and chronic renal failure and was a 30 pack-year ex-smoker. Physical examination revealed a firm cystic mass with a smooth surface, approximately 1 × 1 cm in size, in the right anterior aspect of the tongue (Fig. 1). There was no evidence of cervical lymphadenopathy. Preoperative T1-weighted magnetic resonance imaging (MRI) of the tongue showed a well-defined, ovoid-shaped mass with high-signal intensity, with T1-enhanced MRI showing attenuated signal intensity inside the lesion (Fig. 2). Cervical computed tomography (CT) revealed no cervical lymph node enlargement or other specific findings.

The patient was provisionally diagnosed with a lipoma of the tongue, which was removed surgically. Histopathological examination of the lesion revealed a well-differentiated liposarcoma, also called an atypical lipomatous tumor, with undetermined resection margins. Histologically, the tumor was mainly composed of fatty cells with variation in size and shape and intervening fibrous septum. It also shows characteristic lipoblasts with enlarged indented nuclei and variously sized lipid-containing cytoplasmic vacuoles (Fig. 3).[2]

(Fig. 3). The patient was assessed for metastases by positron emission tomography (PET)-CT, abdominal sonography, and gastroscopy, but no evidence of metastasis was observed. A second operation was performed to completely re-

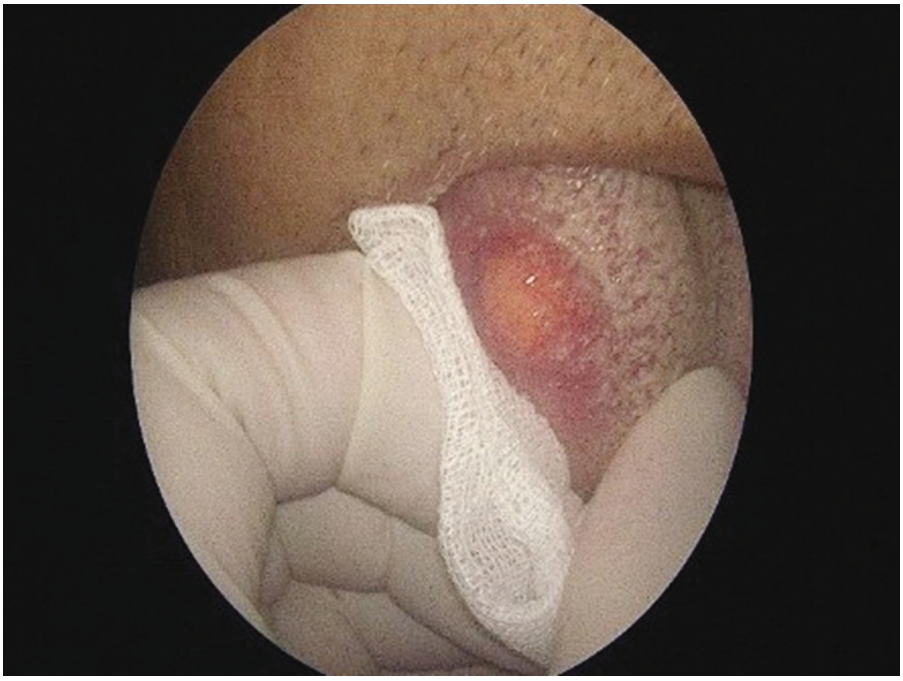

Fig. 1. Laryngoscopic finding, showing a 1 × 1cm sized yellowish mass arising from the right anterior lateral tongue. The mass had smooth surfaces and was well encapsulated.

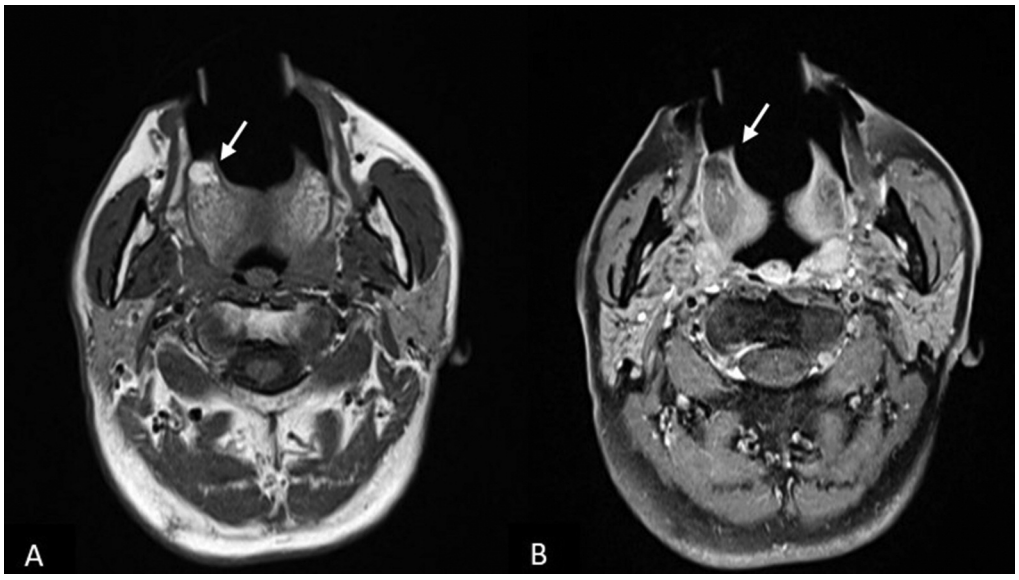

Fig. 2. Preoperative magnetic resonance imaging (MRI) of the tongue mass. (A) Transverse T1-weighted MRI showing a well-defined, ovoid-shaped mass with homogeneous high-signal intensity. (B) Transverse, fat-suppressed T1-weighted, gadolinium-enhanced MRI showing attenuated signal intensity inside the lesion.

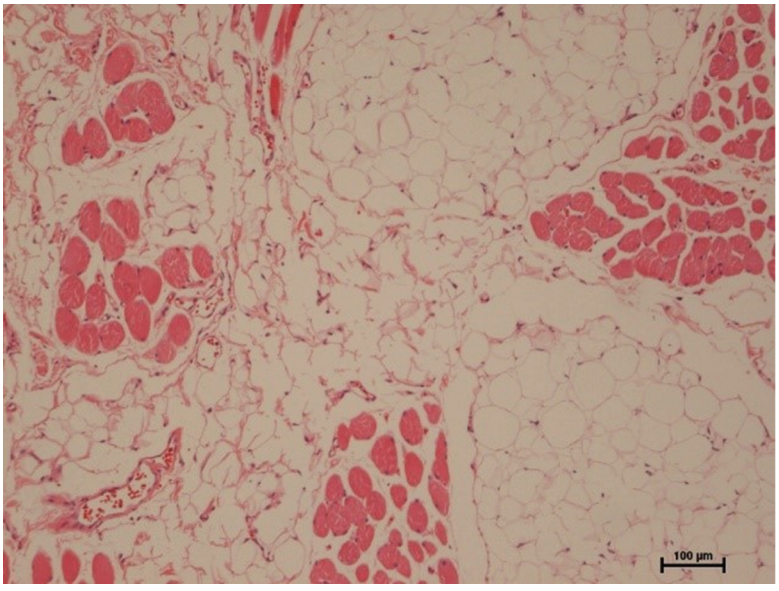

Fig. 3. Pathologic finding. An atypical lipomatous tumor showing mature adipocytes with great variations in size and shape in fibrous stroma (H&E, ×100)

sect the tumor, including safety margins. The postoperative course of the patient was uneventful, with no evidence of tumor recurrence 1 year later.

DISCUSSION

Liposarcomas, first described in 1857, account for approximately 20% of all soft tissue malig-

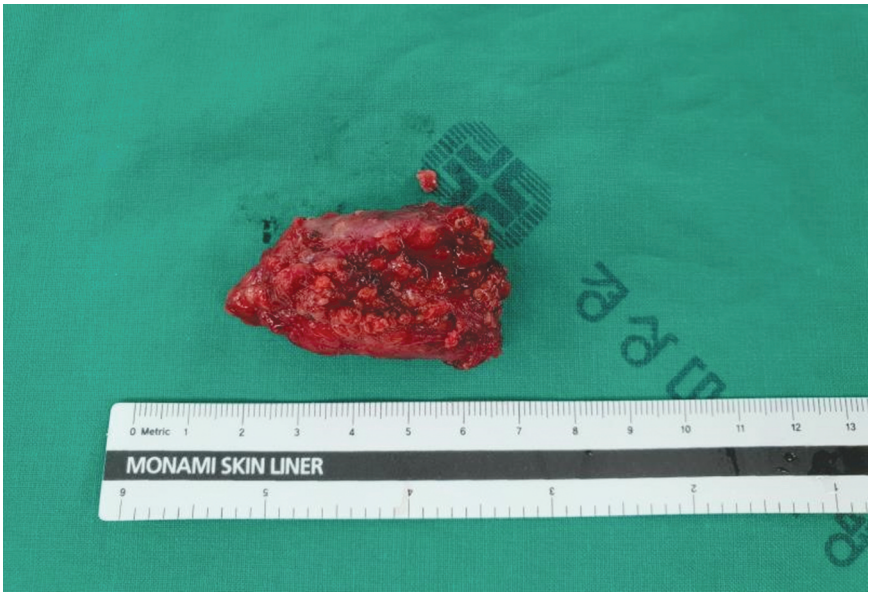

Fig. 4. Lesion removed during the second operation. The specimen was about 6 x 2.5 x 1 cm in size, including a 2 cm safety margin.

nancies and are the most common type of sarcoma in adults. Liposarcomas may arise wherever adipose tissue is normally present, but most occur in deep soft tissues and the retroperitoneum.[3,4] Head and neck liposarcomas are uncommon, constituting 1.8 – 6% of all liposarcomas. Oral liposarcomas are extremely rare. The most common sites in the oral cavity for their occurrence are the oral mucosa (38%), the tongue (33%). the floor of the mouth (7%) and the palate (7%).[4]

Liposarcomas have been classified into several types, including well-differentiated, dedifferentiated, myxoid, pleomorphic and round cell types. Myxoid type is the most common (30 – 50%), followed by well differentiated (20 – 30%), pleomorphic (10 – 25%) and round cell (10 – 15%) types.[5] Well-differentiated liposarcomas, especially in the oral cavity, are predominantly superficial, are of small size, and have clear margins. These tumors rarely metastasize or recur. The WHO has classified well-differentiated liposarcomas as atypical lipomatous tumors.[1] The 5-year survival rate of patients with atypical lipomatous tumors was reported to range from 75% to 100%, compared with a 20% 5-year survival rate in patients with pleomorphic and round cell type tumors.[6]

The distinction between lipoma and atypical lipomatous tumor, however, is a frequent diagnostic dilemma.[2] Although these tumors are firmer and less easily compressed and fixed than benign lipomas, they are not easy to distinguish clinically.[7] The diagnosis of atypical lipomatous tumors is based on pathologic findings, including the presence of lipoblasts, variations in adipocyte size, and adipocytes with atypical and enlarged nuclei.[8,9] Liposarcoma of the tongue can easily be mistaken for benign lipoma, traumatic fibroma, papilloma, generalized gingiva hyperpla-

sia, and pyogenic granuloma.[2]

Surgical resection is the treatment of choice.[7] Wide excision, including safety margins, can significantly reduce recurrence rates.[9] However some groups recommended conservative surgical excision with close and long periods of follow−up instead of radical surgical approach due to a generally favorable prognosis based on the histopathologic subtype, location and clear surgical margins.[10] Local recurrence rates are reported to range from 7% to 30% due to incomplete resection.[4,11] Postoperative radiation therapy may prevent tumor recurrence , especially of radiation sensitive myxoid type tumors. Postoperative radiation therapy is not required for atypical lipomatous tumors if resection margins are tumor free and there is no capsular invasion.[12,13] The patient described in this report did not receive additional radiation therapy because there was no involvement of the capsule after wide excision.

The average size of liposarcomas of the tongue is 0.8 cm, with prognosis being better in patients with tumors < 5 cm in size.[14] By contrast, patients with liposarcomas arising in the retroperitoneum or trunk have a poor prognosis due to the difficulty of surgical resection.[4,15]

Despite its unclear onset, the tumor in this patient was thought to be benign because of its size and characteristics, including its cystic appearance and smooth surface on palpation. Furthermore, imaging suggested that it was a lipoma. However, histologic examination of the lesion suggested that it was an atypical lipomatous tumor. The diagnosis is made histologically. [12] These tumors should be actively treated if their duration is long or unclear and/or they are large in size.

In summary, this report described a patient with a rare atypical lipomatous tumor/well-differentiated liposarcoma of the tongue. The tumor was successfully surgically removed, with no evidence of recurrence at 1-year follow up.

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General Anesthesia for a Patient with *GNE* Myopathy: a case report

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GNE, or bifunctional UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase, myopathy presents with symptoms of foot drop, followed by lower and upper extremity muscle weaknesses and sparing of the quadriceps. Myopathies usually increase the risks of complications related to general anesthesia. The anesthetic management of patients with *GNE* myopathy has not been previously reported. Herein, we report a case of *GNE* myopathy in a 37-year-old woman and discuss anesthetic considerations for elective laparoscopic hysterectomy and bilateral salpingectomy, focusing on the postoperative airway management. We avoided administering neuromuscular-blocking agents and instead used a laryngeal mask airway.

The anesthetic management combining the use of a laryngeal mask airway and desflurane without neuromuscular-blocking agents provided sufficient abdominal and diaphragmatic muscle relaxations for sustaining the pneumoperitoneum for laparoscopic surgery.

Key Words: *GNE* myopathy, Laryngeal mask airway, Neuromuscular Blocking Agents

GNE, or bifunctional UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase, myopathy is also known as a distal myopathy with rimmed vacuoles, quadriceps-sparing myopathy, and hereditary inclusion body myopathy.[1–3] It took 33 years to identify that the same clinical symptoms manifest in all these diseases caused by *GNE* mutations, and thus, they are the same disease.[4] Clinical presentation first starts with foot drop, followed by lower and upper extremity muscle weaknesses but sparing the quadriceps force.[3]

GNE myopathy is a rare disease, and although hyposialylation of muscle glycans is thought to play an essential role, its pathophysiology is not entirely understood and is associated with increased risks of complications of general anesthesia.[5] Induction of general anesthesia is challenging in patients with myopathies, and sufficient preparation and attentive management benefit patients.

Herein, we report a case of *GNE* myopathy in a 37-year-old woman who underwent elective laparoscopic hysterectomy and bilateral salp-

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ingectomy. We reviewed the literature on general anesthesia for adults with *GNE* myopathy. Our key management technique involved the use of a laryngeal mask airway (LMA) and completely avoiding neuromuscular-blocking agents.

CASE

A 37-year-old woman (height, 160 cm; weight, 40 kg; and body mass index score, 15.6 kg/m^2) with *GNE* myopathy was scheduled for laparoscopic hysterectomy and bilateral salpingectomy at our institution. She had no other significant comorbidities. At the age of 20 years, she had developed proximal lower limb weakness and was diagnosed with *GNE* myopathy. The weakness was progressing in all extremities. She had no history of surgery, general or regional anesthesia, or hospitalization for aggravated episodes of *GNE* myopathy.

Blood test results were within normal ranges, and chest X-ray, electrocardiogram, and two-dimensional transthoracic echocardiogram were unremarkable. Muscle strength in the limbs was reduced by 2/5 according to the Medical Research Council scale for muscle strength.[6] The pulmonary function test (PFT) showed a severe restrictive pattern. Preoperatively, glycopyrrolate 0.2 mg was injected intramuscularly, and a 20-gauge intravenous cannula was inserted into the dorsum of the left hand. The patient was placed in the supine position, and all basic monitors were applied. She had a preoperative blood pressure of 120/74 mmHg, heart rate of 66 bpm, and peripheral capillary oxygen saturation of 100% while breathing room air. The bispectral index (BIS, XP version 4.1; Aspect Medical Systems, Newton, MA, USA) was used to monitor the depth of anesthesia. For anesthesia induction, 1% propofol 80 mg and 1% lidocaine 20 mg were administered, and remifentanil (0.05 – 0.50 μg/kg/h depending on the patient's vital signs and the surgical procedure) was infused continuously. No neuromuscular blocking agents were used. After loss of consciousness was confirmed, LMA (LMA Supreme^{TM}, Teleflex, Ireland) was inserted. Desflurane (5 – 6 vol%) was used for maintenance, and fraction of inspired oxygen was maintained at 0.6 (mixture of medical air and oxygen).

A nasal temperature probe was inserted, and temperature was maintained above 36°C. Invasive positive pressure ventilation was maintained throughout the operation with time-cycled, volume-controlled ventilation at a tidal volume of 320 mL and respiratory rate of 10 – 14 breaths/min. End-tidal CO_2 partial pressure was maintained at 34 – 37 mmHg. Peak inspiratory pressure did not exceed 20 mmHg throughout the procedure. Oral suction was performed through the port in the LMA using a small nasogastric tube for infants. Anesthesia lasted 75 min, and the LMA was removed uneventfully in the operating room. A total of 300 mL of crystalloid solution was administered and no other additional medication were needed (e.g. vasopressors, inotropic agents etc.). There were no incidents of unwanted movement, coughing, difficulty in ventilation, difficulty achieving or maintaining pneumoperi-

toneum, or surgical technical difficulty resulting from the omission of neuromuscular blockade. During laparoscopic procedure, CO_2 insufflation pressure was kept at 12 - 13 mmHg, and no additional CO_2 pressure was required to achieve adequate pneumoperitoneum. Planned postoperative analgesia included a single intravenous bolus of 4 mg oxycodone with 4 mg nefopam.

After observation in the recovery room for 1 h, with no episodes of desaturation, she was discharged from the hospital on postoperative day 5 after achieving good pain control with oral analgesics and without complications from surgery or anesthesia.

DISCUSSION

GNE myopathy is a rare distal myopathy that progresses slowly and is caused by mutations in *GNE*. Although respiratory dysfunction has rarely been reported in patients with *GNE* myopathy, a retrospective review by Makdoka et al. revealed that *GNE* myopathy can cause severe respiratory failure.[7] Considering our patient's severely restrictive PFT pattern and *GNE* myopathy as the disease entity, we suspected that intentional respiratory muscle paralysis with neuromuscular blocking agents would lead to the requirement of mechanical ventilation support. Our main anesthetic goal was to maintain adequate anesthesia without using any neuromuscular blocking agent. Peripheral nerve stimulation is used to monitor dose-response to neuromulscular blocking agent and we concluded that it was not only unnecessary but also regarding progression of disease in this case, already affected distal myopathic muscle was unreliable to evaluate laryngeal or central respiratory muscle strength. In other patients, when the laparoscopic method is used for abdominal surgery, pneumoperitoneum with CO_2 is necessary, and during that time, adequate abdominal and diaphragmatic muscle relaxations are crucial. Volatile anesthetics, such as sevoflurane and desflurane, have some muscle relaxation properties, and therefore, reduced doses of neuromuscular blocking agents are required during general anesthesia.[8] Wiklund et al. revealed that halothane, sevoflurane, and desflurane relaxed the smooth muscles in the airways through the inhibition of the cholinergic neuroeffector transmission.[8] Hence, we used propofol for fast anesthesia induction and desflurane for maintenance.

In patients with general central myopathy (e.g. Duchenne's muscular dystrophy, myotonic disorders, congenital myopathies, etc.), anesthetic management requires avoiding drugs such as volatile anesthetics and succinylcholine, which are associated with malignant hyperthermia (MH)-like reactions and severe hyperkalemia. However, it is well understood that MH-like reactions in most myopathies are a separate disease entity that follow a different pathophysiologic pathway. The most common reason for congenital myopathies is the *RYR1* mutation, and only some cases are linked with MH susceptibility. Congenital MH-linked myopathies include central core disease, King-Denborough syndrome, multi/minicore disease, nemaline myopathy, and

Evans myopathy, but not *GNE* myopathy.[5] In specific myopathies with a high risk of anesthesia-induced rhabdomyolysis (AIR) or MH, volatile anesthetics and 1neuromuscular blocking drugs should be used with caution or avoided completely in some cases. However, muscular dystrophies, myotonia, and mitochondrial myopathies have little associations with malignant hyperthermia or AIR. In general, both propofol and desflurane are well tolerated in patients with distal muscular dystrophies, however, there are some concerns associated with the propofol-infusion syndrome when used as a maintenance agent.[6] In distal skeletal muscle-related GNE myopathies, both desflurane and induction-dose propofol can be safely used without causing unnecessary complications. However, considering the surgical method of involving laparoscopes, some muscle relaxation is necessary, thus validating the use of volatile anesthetics rather than propofol for maintenance of anesthesia.

In the laparoscopic method, endotracheal intubation is preferred to LMA insertion due to elevated abdominal pressure and the disadvantage of managing adequate airway. But it would require adequate laryngeal and tracheal muscle relaxation with neuromuscular blocking agent which would disarticulate from our goal not to use any previously mentioned drugs. This can explain our choice to use LMA rather than endotracheal tube and also we interpreted that the severely restricted pulmonary function of our patient could have caused the absence of coughing or sudden movement during surgery.

In summary, combining the use of LMA and desflurane for anesthesia maintenance, without any neuromuscular blocking agent, abdominal and diaphragmatic muscle relaxations were sufficient for sustaining pneumoperitoneum for laparoscopic surgery.

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Klebsiella pneumoniae-induced Liver Abscess Complicated with Septic Pulmonary Embolism in a Non-diabetic Adult

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A 72-year-old non-diabetic man was admitted to the intensive care unit because of liver abscess, cholecystitis, and septic shock. He underwent percutaneous catheter drainage and received intravenous antibiotics. Shock was improved, and the patient's fever subsided. *Klebsiella pneumoniae* was isolated in blood and bile cultures. However, he suddenly developed dyspnea and oxygen desaturation. Chest computed tomography scan revealed multifocal ground-glass opacities with consolidation with peripheral preponderance. Appropriate antibiotic therapy was provided for 2 weeks. The patient recovered fully, and cholecystectomy was then performed. Herein, we report a case of *K. pneumoniae*-induced liver abscess complicated with septic pulmonary embolism in a non-diabetic patient.

Key Words: Klebsiella pneumonia, Liver abscess, Pneumonia

Liver abscess is caused by various strains of bacteria and is characterized by upper right abdominal pain and fever. *Escherichia coli* is the most common causative strain. However, *Klebsiella pneumoniae* was recently considered as the most common causative organism in Korea.[1] Hepatic abscesses caused by *K. pneumoniae* are associated with diabetes mellitus and can cause metastatic infections in different organs, which include liver abscesses, spondylitis, and endophthalmitis.[2,3] The prevalence of *K. pneumoniae*-induced septic pulmonary embolism complicated with liver abscess is low, with a rate of 4.5%-6% among 86% of diabetic patients. It can manifest in various forms, such as multiple nodules, pleural effusion, and ground-glass opacity (GGO) with or without the involvement of bilateral cavities on computed tomography (CT) scan.[4,5] The prognosis of patients with septic pulmonary embolism is poor. Thus, such condition must be considered, and early diagnosis and differentiation should be performed.[4-7]

Herein, we report a case of multiple septic pulmonary embolism with a time difference in a non-diabetic patient who was on antibiotic treatment for *K. pneumoniae* liver abscess and who

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had percutaneous drainage for early-stage cholecystitis.

CASE

A previously healthy 72-year-old man visited the emergency room because of fever and right upper abdominal pain. He had been diagnosed with hypertension 10 years previously and was on medication for hypertension. He had nonspecific family history and had stopped smoking 5 years ago (50 pack-years).

Physical findings were as follows: blood pressure, 117/61 mmHg; pulse rate, 92 beats/minute; respiratory rate, 21 cycles/minute; and body temperature, 38.7°C. Moreover, the patient was alert, and his breathing sounds were normal. On the basis of an abdominal examination, the patient had right upper abdominal tenderness, and Murphy sign was observed.

The peripheral blood test results were as follows: white blood cell count, 9,900 mm^3 (neutrophil: 98.0%, lymphocyte: 1.8%, and monocytes: 0.2%). hemoglobin level, 11.3 mg/dL; platelet count, 128,000/mm^3. Meanwhile, serum biochemistry was performed, and the results were as follows: aspartate aminotransferase level,156 IU/L; and alanine aminotransferase level, 156/58 IU/L, alkaline phosphatase level, 174 U/L; γ-glutamyl transpeptidase level, 280 IU/L; total bilirubin level, 2.10 mg/dL; lactate dehydrogenase level, 872 U/L; blood glucose level, 87 mg/day; glycated hemoglobin (HbA1c) level, 6.0%; and C-reactive protein level, 17.4 mg/dL. The patient tested negative for hepatitis B surface antigen and anti-hepatitis C Ab and positive for anti-HB antibody. *K*. pneumoniae was detected in two pairs of blood culture and one pair of bile culture.

Simple chest radiography and chest CT scan were performed at the emergency room, and no specific findings were obtained, except for emphysema and bronchitis (Fig. 1). Moreover, abdominal CT scan revealed the presence of a liver abscess in segment 4 (4.5 cm × 3.7 cm) and a dilated gallbladder with diffuse inflammatory thickening (Fig. 2).

The blood pressure of the patient immediately dropped to 80/40 mmHg after the emergency room visit. However, it normalized after hydration and administration of vasopressor. Percutaneous drainage was performed for cholecystitis, and the patient was admitted in the intensive care unit (ICU) due to liver abscess. Treatment with ciprofloxacin and metronidazole was initiated. On the second day of hospitalization, the patient's blood pressure remained within normal range even without the use of a vasopressor. Thus, he was transferred to the general ward.

However, on the fourth day, the patient presented with difficulty breathing and fever, which subsequently worsened. The patient's oxygen saturation decreased to 89% even with high-flow oxygen therapy (fraction of inspired oxygen [FiO2]: 80%, flow: 50 L). Multiple chest GGO and consolidation were observed on chest CT scan. The patient was again admitted to the ICU because of suspected nonspecific pneumonia. On the fourth day, *K. pneumoniae* was identified in the blood culture, and antibiotic susceptibility

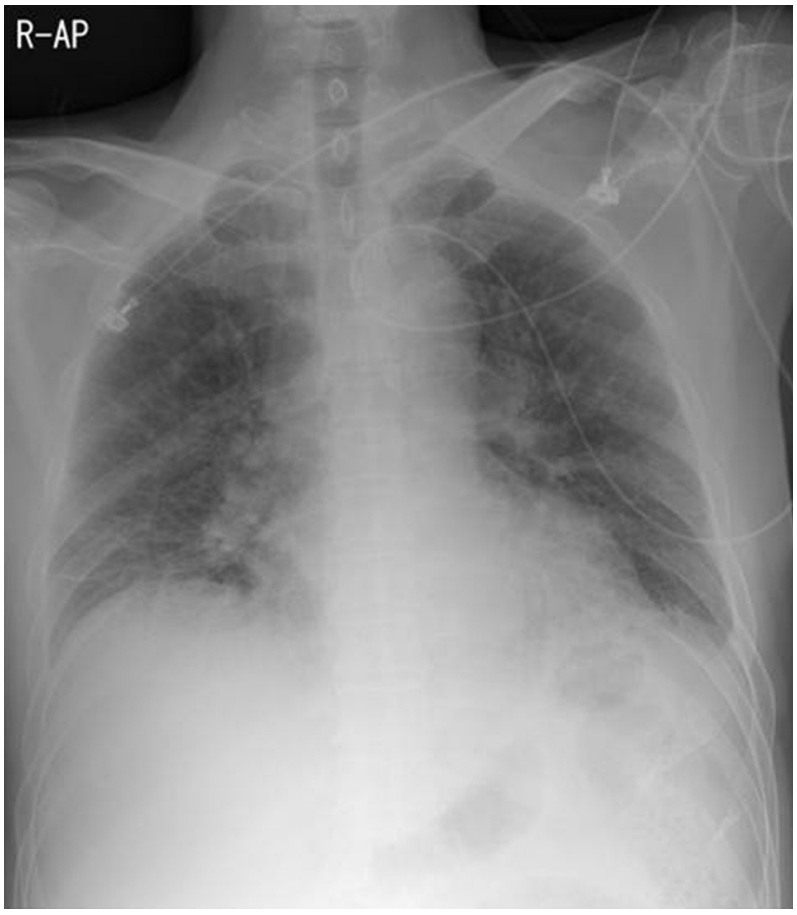

Fig. 1. Initial chest radiography revealed subtle linear opacity in both lower lung fields.

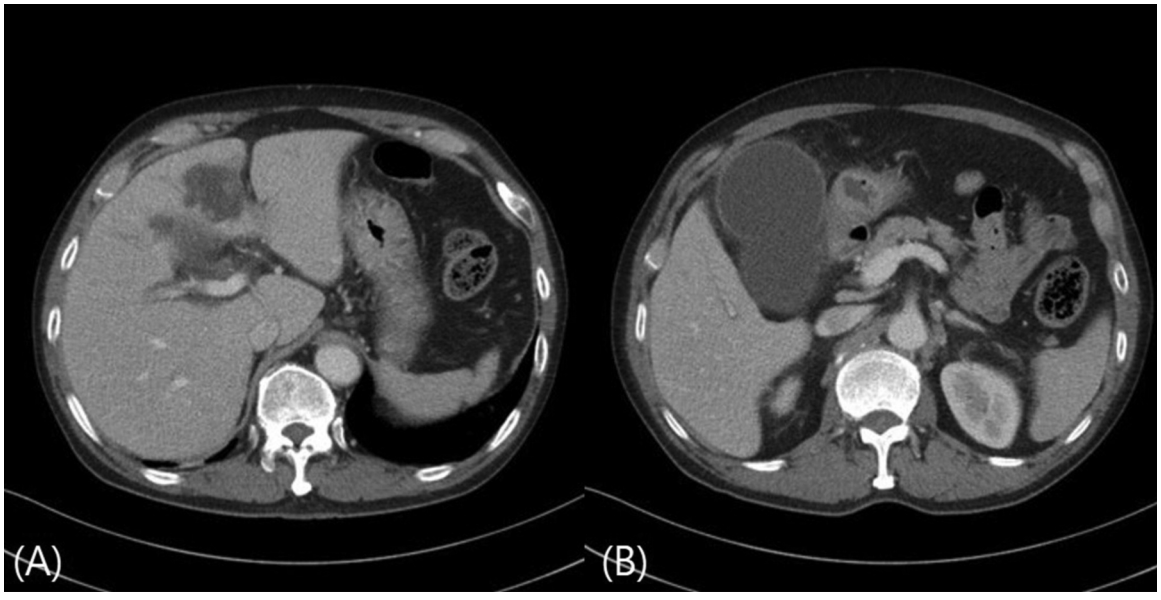

Fig. 2. Abdominal computed tomography scan revealed a 4.5-cm liver abscess in segment 4 of the liver (A) and a dilatated gallbladder with diffuse inflammatory thickening (B).

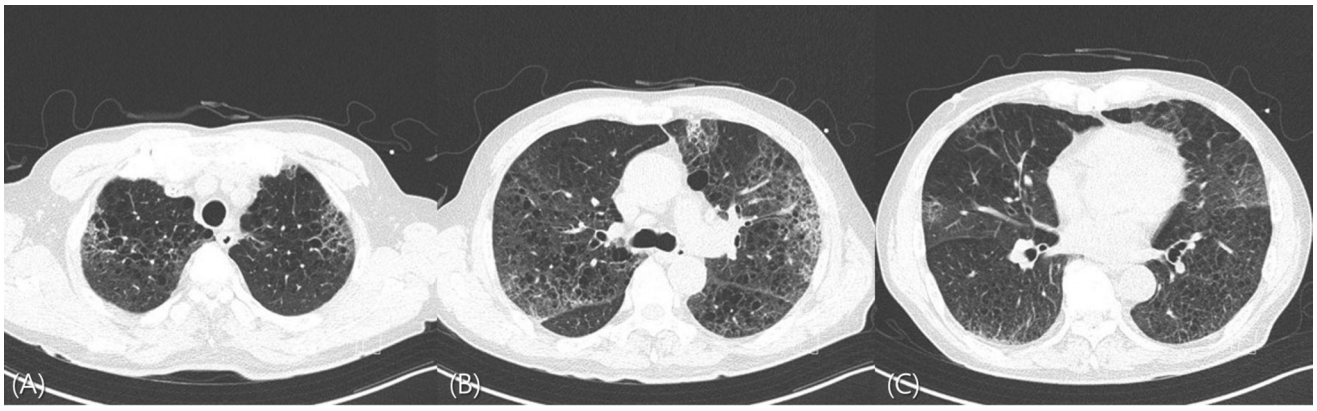

Fig. 3. Chest computed tomography scan revealed multifocal ground-glass opacities with consolidation in both upper lung fields (A), the right middle lung (B), and the lingular segment of the left upper lung with peripheral preponderance (C).

tests revealed susceptibility to all antibiotics, except ampicillin. However, as the patient's condition worsened and fever persisted, the antibiotics were changed to piperacillin/tazobactam and metronidazole.

On the sixth day of hospitalization, the patient did not present with fever, and *K. pneumoniae* was identified in one pair of bile culture. On the seventh day, his oxygen demand decreased (FiO2: 50%, flow: 35 L). Chest CT scan was then performed, and results revealed bilateral multiple GGO and consolidation (Fig. 3). The patient's C-reactive protein level decreased to 5.9 mg/dL. The presence of bacteria was not identified in the blood culture performed on the fifth day and thereafter.

The patient did not complain of conjunctival bleeding or decreased visual acuity. However, he was referred to an ophthalmologist for the examination for endophthalmitis caused by *K*. pneumoniae. Other than cataracts, no specific findings were observed. After the administration of piperacillin/tazobactam and metronidazole, all clinical symptoms improved. The patient was prescribed oral moxifloxacin as home medication and was then discharged. Since then, the patient has undergone a cholecystectomy in the department of surgery. The patient is currently being followed up in the outpatient department.

DISCUSSION

Pyogenic liver abscess is a common infection that occurs in the abdominal cavity and is often transmitted directly from biliary tract infections and, sometimes, bacteremia. However, the other causes are unknown.[2] *E. coli* was found to be a common causative organism in Korea before the 1980s; since then, the organism has been considered a common causative agent.[1] K. pneumoniae was identified as an emerging cause of community-acquired liver abscess in Korea.[8]

K. pneumoniae liver abscess is frequently associated with septic metastatic lesions, endoge-

nous endophthalmitis, cerebral abscess, meningitis, and infectious spondylitis.[9-12] An invasive *K. pneumoniae* liver abscess syndrome is defined as *K.* pneumoniae-induced liver abscess that is accompanied by metastatic infections involving an organ other than the liver.[2] Among these infections, septic pulmonary embolism is not common, and the diagnosis is often delayed. In a study conducted in Taiwan, the common symptoms of septic pulmonary embolism associated with *K. pneumoniae* liver abscess were fever and dyspnea, and chest CT scan revealed nodules, pleural effusions, and wedge-shaped lesions involving the margins of the lungs, with or without the cavities.[4] In this patient, K. pneumoniae was not identified in sputum culture, but in other studies, K. pneumoniae was confirmed in sputum in only 3 out of 9 patients with septic embolism accompanying K. pneumonia liver abscess.[13] And another study showed negative findings in sputum culture (n = 9) and endobronchial culture (n = 5) among 14 patients.[4] Thus, the sensitivity of sputum culture is found to be around 50%,[14] so the imaging work up is more emphasized in pulmonary septic embolism.

Diabetes is a risk factor for *K. pneumoniae*-induced liver abscess, and about 80% of patients with diabetes due to an underlying disease present with *K. pneumoniae* liver abscess.[15] Patients with uncontrolled blood sugar levels are at higher risk of gas-forming liver abscesses, liver abscesses of unknown origin, and metastatic infections than those with controlled blood sugar levels. Liver abscesses of unknown origin and metastatic infections are more common in groups with poor blood sugar control (HbA1c > 10%). HbA1c levels and abscess size < 5 cm are independent risk factors for the metastatic complications of *K. pneumoniae* liver abscess.[16] This patient was an ex-smoker and had an emphysematous change in the lungs, and it was thought that the appearance of K. pneumoniae bacteremia due to liver abscess with cholecystitis caused micro embolism, resulting in septic pulmonary embolism.

Septic pulmonary embolism can be identified based on the presence of a nodule in the parenchyma, including the pulmonary margins and various cavities and blood vessels supplying them, and heterogeneous, wedge-shaped lesions in the pleura.[4,13] However, the differential diagnoses of several pulmonary nodules include tuberculosis, fungal infections, and tumors. The disappearance of pulmonary nodules after proper antimicrobial therapy is an indication that the diagnosis of septic pulmonary embolism is correct. However, other examinations should also be performed to rule out other potential causes of pulmonary lesions.

In this case, the non-diabetic patient was diagnosed with liver abscess associated with cholecystitis. *K. pneumoniae* was identified in the blood and bile cultures. Based on the initial chest radiography, there were no specific findings, except for emphysema and bronchitis. However, in the general ward, the patient presented with hypoxemia and dyspnea while on antibiotic treatment. Thus, chest CT scan was

performed and revealed multiple GGO with consolidation. The patient was treated in the ICU because of suspected metastatic lung infection. The sputum cultures did not reveal the presence of *K. pneumoniae* probably because the test was performed after antibiotic treatment and the probability of culturing was low.

The mortality rate of septic pulmonary embolism associated with liver abscess is as high as 14%.[4] Therefore, when patients with *K. pneumoniae* liver abscess complain of fever and respiratory symptoms, the possibility of septic pulmonary embolism should be considered. Thus, active evaluation, such as performing imaging and sputum tests, must be performed, and treatment should be provided.

Conflicts of interest

The authors have no conflicts of interest.

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Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany(NY): Delmar Publishes; 1996

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Disaia PJ, CreasmanWT. The adnexal mass and early ovarian cancer. In: Disaia PJ, CreasemanWT, editors. Clinical gynecologic oncology. 5th ed. St.Louis: Mosby- Year Book; 1997. p.253-61.

5) **Conference/meeting presentation**

Morrow GR, Ryan JL, Kohli S. Modafinil for persistent post-treatment fatigue: an open label study of 82 women with breast cancer.MASCC international symposia 2006, Abstract 11-070.

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AMA: helping doctors help patients [Internet]. Chicago:

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