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Effects of White-coat Hypertension on Heart Rate Recovery and Blood Pressure Response during Exercise Test

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Objectives: White-coat hypertension is defined as high blood pressure (BP) on clinical assessment but normal BP elsewhere or on ambulatory measurement. Autonomic dysfunction may be one of the mechanisms causing white-coat hypertension. Slowed heart rate recovery and excessive BP response during exercise test are associated with autonomic dysfunction. The purpose of this study was to determine the association between white-coat hypertension and abnormal autonomic nervous system response.

Methods: We assessed 295 patients stratified into three groups via 24hr ambulatory BP monitoring, following 2017 ACC/AHA guidelines : normal BP group, white-coat hypertension group, and a hypertension group. We analyzed medical history, blood test, echocardiography, 24hr ambulatory BP monitoring, and exercise test data.

Results: There was no difference in basement characteristics and echocardiography among the groups. Blunted heart rate recovery of each group showed a significant difference. Control group had 0% blunted heart rate recovery, but 33.3% in white coat group and 27.6% in true hypertension group ($P < 0.001$). Also, in the control group, 4.5% showed excessive BP response, but 31.5% in the white coat hypertension group and 29.3% in the true hypertension group ($P < 0.001$). Excessive BP response during the exercise test or blunted heart rate recovery, which is an indicator of autonomic nervous system abnormality, was more common in the hypertensive group and white-coat hypertension group than in the normal BP group.

Conclusions: These results confirmed that white-coat hypertension has an autonomic nervous system risk. Therefore, white-coat hypertension can be a future cardiovascular risk factor.

Key Words: Autonomic dysfunction, Exercise test, White-coat hypertension

Hypertension is one of the most common diseases that affects about 30% of the population over the age of 30 years in Korea.[1] The prevention, diagnosis, and management of hypertension is very important because it is closely related to cerebrovascular disease and cardiovascular disease, which accounts for several deaths in Korean adults.[2]

Patients with normal blood pressure (BP) during rest may occasionally stop testing their BP level due to excessive elevation in systolic BP (SBP) during exercise testing. One of the under-

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lying mechanisms of the hypertensive response to exercise is the increase in the sympathetic response.[3] Increased sympathetic activity is known to be one of the mechanisms underlying the exaggerated BP response to exercise (EBPR).[4]

EBPR is associated with a higher rate of hypertension than normal, and is associated with increased cardiovascular mortality.[5] The risk of hypertension can be confirmed through the exercise test.[6] In addition, exercise capacity and heart rate recovery (HRR) were significant predictive factors of the relative risk of death.[7] Measurement of HRR is a simple non-invasive procedure analyzing the autonomic nervous dysfunction.[8,9] An earlier study showed that a blunted HRR, which is defined as a decrease in heart rate (HR) of less than 12 beats/min from peak exercise to 1 min into recovery, is a powerful predictor of overall mortality.[7,10]

White-coat hypertension is defined as high BP on clinical assessment but normal BP elsewhere or on ambulatory measurement. In this case, ambulatory BP monitoring and home BP monitoring are recommended.[11] Autonomic dysfunction may be one of mechanisms causing white-coat hypertension.[12] Its association with HRR and EBPR in white-coat hypertension patients has not been well studied.

We aimed to investigate the association between HRR and EBPR in patients with white-coat hypertension.

MATERIALS AND METHODS

This is a cross-sectional, single center, case control study. We retrospectively reviewed 295 patients who underwent Treadmill test and 24-hour ambulatory BP monitoring (ABPM) between January 2008 and February 2015. Inclusion criteria were: 18–80 years of age, normal renal function, and for women to be on a regular menstrual cycle. Exclusion criteria were: any systemic disease such as significant liver disease, neurologic disorders or malignant disease, or secondary hypertension.

Patients were classified according to the ABPM, following diagnostic criteria suggested by the 2017 ACC/AHA guidelines.[13] Hypertension group was meeting one or more of these criteria; a 24-hour mean of 125/75 mmHg or above, daytime (awake) mean of 130/80 mmHg or above or Nighttime (asleep) mean of 110/65 mmHg or above. White coat hypertension is defined as having elevated clinic blood pressure, at or above threshold for hypertension without elevated out-of-office blood pressure, below threshold for hypertension. Patients not included in the two groups were classified as normotensive control. After all, patients were classified as 174 patients with true hypertension, 54 patients with white-coat hypertension, and 67 normotensive controls were included.

Demographic characteristics recorded at the first visit included age, sex, height, weight, current medications, smoking history, and other comorbidities. Blood was drawn for the measurement of total serum cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol, triglycerides, blood glucose, creatinine, uric acid, and high sensitivity

C-reactive protein (Hs-CRP). Body mass index (BMI) was calculated as the ratio of weight in kilograms to height in square meters. This study was approved by the Kosin University International Review Board.

- BP measurement and 24-h ambulatory BP monitoring

Office BP measurements were performed twice at 5-min intervals using a mercury sphygmomanometer. Noninvasive 24-h ABPM was performed on each patient's non-dominant arm using an automatic oscillometric device (TONOPORT V, PAR Medizintechnik, Berlin, Germany) on a normal working day. Patients were asked to refrain from performing fast exercises. All subjects were instructed to rest or sleep between 10:00 PM and 7:00 AM (nighttime) and continue their usual activities between 7:00 AM and 10:00 PM (daytime). The accuracy of the device was checked against the standard auscultatory method of measuring BP to ensure that the difference in BP measurements between methods did not exceed 5 mmHg. The device was set to obtain BP readings at 20-min intervals during the daytime and at 40-min intervals during the nighttime. Only 24-h recordings that included at least 80% successful recordings were accepted as valid. Each ABPM dataset was first automatically scanned to remove artifactual readings according to preselected editing criteria. The following ABPM parameters were evaluated: 24-h mean SBP and diastolic BP (DBP) levels, daytime mean SBP and DBP levels, nighttime mean SBP and DBP levels, and BP variability assessed with standard deviation (SD).

- Treadmill test

All patients underwent symptom-limited exercise stress testing (GE CASE T2100; GE Medical Systems, Milwaukee, WI, USA) according to the protocol by Bruce et al..[14] BP was measured using an automated BP monitor (Suntech Tango; Suntech Medical, Morrisville, NC, USA) throughout the treadmill test using the same arm as was used to measure the resting BP. Twelve-lead electrocardiography was monitored continuously and printed at a paper speed of 25 mm/s; measurements of HR and BP were recorded at the end of each 3-min stage at peak exercise and at 1-min and 2-min intervals throughout recovery.

Treadmill test was continued until the participants felt intolerable fatigue or their HR exceeded 95% of estimated maximal HR (220 bpm, age). The total exercise time was also recorded. EBPR was defined as the peak exercise SBP of $\geq$ 210 mmHg in men and $\geq$ 190 mmHg in women.[15] Functional capacity was estimated in metabolic equivalents (METs) on the basis of speed and grade of the treadmill.[16] During the recovery phase, the subjects continued to walk for 60s at a speed of 1.5 mph, and then they sat down for 3min with continued monitoring of BP, HR, and heart rhythm. HRR was defined as peak heart rate minus heart rate after a 1-min recovery; abnormal HRR was defined as $\leq$ 12 beats/min.[17]

- Echocardiographic measurement

Standard 2-dimensional echocardiography was performed on all subjects lying in the left lateral

decubitus position using a 3.5-MHz transducer (Philips iE33, Philips Medical Systems, Bothell, WA, USA) and the echocardiography examiners were blinded to patient information. Measurements of thickness of the interventricular septum and posterior wall, diameter of the left ventricle (LV) cavity, and the LV mass index (LVMI) were performed according to criteria outlined by the American Society of Echocardiography.[18] Pulsed wave Doppler of transmitral LV inflow was performed in the apical four-chamber view with the sample volume placed at the level of the mitral valve tips; doppler variables were analyzed during three consecutive beats. The following measurements of global LV diastolic function were determined: peak early (E) and late (A) diastolic mitral flow velocity, E/A ratio, and early (Ea) diastolic mitral annular velocity.

- Statistical analysis

Statistical analyses were performed using the commercially available computer program SPSS 18.0 for Windows (IBM, Chicago, IL, USA). Data are presented as mean ± standard deviation for continuous variables and percentage (%) if the data are categorical. The Mann–Whitney U test was used for continuous variables and the Chi-square test was used for categorical data. The normality of data was tested using the Kolmogorov–Smirnov test. For all tests, the significance level was set to $P < 0.05$.

RESULTS

1. Characteristics & Medical history

From January 2008 to February 2015, 295 patients were enrolled in this study. Patients were divided into control, white-coat hypertension, and true hypertension groups.

At baseline, compared to the normotensive control group, the white-coat hypertension group showed higher BMI, SBP, and DBP. BMI was lowest in the control group at 23.2 kg/m2 and highest at 24.9 kg/m^2 in the true hypertension group ($P = 0.001$). SBP/DBP was lowest in the control group and highest in the white-coat group ($P < 0.001$). There was no difference in age, sex, heart rate, current smoking, diabetes, and dyslipidemia among the groups (Table 1).

There was no statistically significant difference in medical history with regard to aspirin, RAS blocker, calcium channel blocker, diuretics, beta blocker, and statin intake.

2. Laboratory test

In laboratory data, uric acid, eGFR, Hs-CRP, WBC, and Platelet levels were similar between the groups. Mean total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides were the highest in the true hypertension group, but the difference was not significant.

3. 24-h ambulatory HR & BP monitoring

24-h ambulatory BP monitoring was done. As a result, 24-h heart rate and daytime and night time heart rate were higher in the true hypertensive group than in the control and white hypertensive groups ($P = 0.001$). Additionally, the 24-hour systolic blood pressure/diastolic blood pressure was 142/91 in the true hypertension group, but 119/75

Table 1. Baseline characteristics

	Control (n = 67)	White coat hypertension (n = 54)	True hypertension (n = 174)	Anova *P*
Age, years	52.3 ± 12.0	54.2 ± 13.6	51.7 ± 14.8	0.522
Male gender, n(%)	30 (44.8)	25 (46.3)	99 (56.9)	0.038
Body mass index, kg/m^2	23.2 ± 2.81	24.8 ± 3.48	24.9 ± 3.41	0.001
Systolic BP, mmHg	123.4 ± 11.8	140.9 ± 14.5	136.2 ± 18.7	< 0.001
Diastolic BP, mmHg	72.5 ± 10.6	86.1 ± 18.7	82.33 ± 14.1	< 0.001
Heart rate, /min	64.3 ± 12.7	63.6 ± 12.3	67.3 ± 11.2	0.059
Current smoking, n(%)	6 (9.1)	4 (7.5)	25 (14.7)	0.409
Diabetes, n(%)	5 (7.5)	3 (5.6)	14 (8.0)	0.830
Dyslipidemia, n(%)	11 (26.8)	9 (25)	62 (40.8)	0.086
Aspirin, n(%)	16 (23.9)	12 (22.2)	31 (18.0)	0.562
RAS blockade, n(%)	18 (26.9)	12 (22.2)	32 (18.6)	0.365
Beta blocker, n(%)	17 (25.4)	12 (22.2)	33 (19.2)	0.562
CCB, n(%)	15 (22.4)	9 (16.7)	37 (21.5)	0.699
Diuretics, n(%)	7 (10.4)	1 (1.9)	12 (7.0)	0.175
Statin, n(%)	22 (32.8)	10 (18.5)	35 (20.3)	0.081

SBP : systolic blood pressure, DBP : diastolic blood pressure, RAS : renin angiotensin system, CCB : calcium channel blocker

and 122/75 in the control and white-coat hypertension groups respectively. This represents a significant difference in BP ($P < 0.001$). Day time SBP, DBP, SBP-SD, DBP-SD, MBP, and MBP-SD showed meaningful differences between control, white-coat hypertension, and true hypertension groups. All results were highest in the true hypertension group, and the same appeared in the night time ($P < 0.001$) (Table 2).

4. Echocardiography

Echocardiography was performed on all patients and compared according to hypertension type. Ejection fraction (EF), diastolic left ventricular internal dimension (LVIDd), systolic left ventricular internal dimension (LVIDs), left atrium (LA) diameter, LA volume, E velocity, A velocity, and E/Ea shows no significant difference. The white-coat hypertension and true hypertension groups have significantly higher epicardial fat thickness value, and the left ventricle posterior wall diameter on echocardiography was thicker in the true hypertension group than in the control and white hypertensive groups ($P < 0.001$).

Table 2. Clinicopathogenic features of papillary thyroid carcinomas patients analyzed in this study

	Control (n = 67)	White coat hypertension (n = 54)	True hypertension (n = 174)	ANOVA *P*
24HR	69.98 ± 8.08	68.11 ± 8.16	73.57 ± 10.92	0.001
24HR-SD	15.42 ± 6.58	15.39 ± 6.04	14.74 ± 6.25	0.726
Daytime HR	73.42 ± 8.59	70.01 ± 13.19	76.88 ± 11.66	< 0.001
Nighttime HR	60.89 ± 7.70	59.02 ± 8.18	64.20 ± 10.71	0.001
Daytime HR-SD	15.98 ± 7.63	16.49 ± 8.83	14.29 ± 6.73	0.104
Nighttime HR-SD	6.65 ± 4.71	6.15 ± 4.69	8.40 ± 5.72	0.002
24h SBP	118.59 ± 7.74	121.63 ± 7.77	142.76 ± 12.66	< 0.001
24hr DBP	74.84 ± 4.75	75.19 ± 5.00	91.00 ± 9.85	< 0.001
24hr SBP-SD	13.06 ± 3.52	14.28 ± 4.09	15.74 ± 3.86	< 0.001
24hr DBP-SD	10.72 ± 3.24	11.03 ± 3.56	13.63 ± 3.90	< 0.001
24hr MBP	89.26 ± 5.16	90.30 ± 5.60	107.91 ± 10.12	< 0.001
24hr MBP-SD	11.16 ± 3.33	11.58 ± 3.56	14.03 ± 3.50	< 0.001
Daytime SBP	120.87 ± 7.78	124.15 ± 8.09	145.80 ± 12.73	< 0.001
Daytime DBP	76.93 ± 4.81	77.18 ± 5.65	93.68 ± 10.26	< 0.001
Daytime SBP-SD	12.56 ± 4.09	13.44 ± 4.44	14.61 ± 4.43	0.004
Daytime DBP-SD	10.64 ± 3.94	10.37 ± 4.18	12.87 ± 4.57	< 0.001
Daytime MBP	91.10 ± 5.36	92.54 ± 6.07	110.73 ± 10.38	< 0.001
Daytime MBP-SD	10.74 ± 3.96	10.82 ± 4.07	12.73 ± 4.26	< 0.001
Nighttime SBP	111.07 ± 15.47	112.22 ± 19.31	134.19 ± 15.23	< 0.001
Nighttime DBP	70.13 ± 6.15	69.47 ± 6.46	83.34 ± 10.89	< 0.001
Nighttime SBP-SD	10.34 ± 3.04	10.95 ± 3.39	12.51 ± 4.11	< 0.001
Nighttime DBP-SD	8.51 ± 2.88	8.51 ± 3.39	10.50 ± 3.84	< 0.001
Nighttime MBP	83.93 ± 6.93	86.46 ± 17.15	100.06 ± 11.63	< 0.001
Nighttime MBP-SD	8.75 ± 2.76	8.94 ± 3.3	10.69 ± 3.72	< 0.001

All values are presented as the mean ± SD. hr : hour, HR : heart rate, SD : standard deviation, SBP : systolic blood pressure, DBP : diastolic blood pressure

5. Exercise test

Compared to the normotensive group, there is no difference in exercise time, rest heart rate, max heart rate, QT, and QTC. However, HRR was significantly lower in the white-coat hypertension and true hypertension groups when compared to

the normotensive group ($P < 0.001$). HRR between groups showed a significant difference. The control group had 0 blunted HRR, but 33.3% (18 patients) in the white-coat group and 27.6% (48 patients) in the true hypertension group. This shows that the prevalence of blunted HRR was significantly higher in the white-coat hypertension and true hypertension groups than the normotensive group (Table 3).

In the control group, 4.5% (3 patients) showed EBPR, but 31.5% (17 patients) in the white-coat hypertension group and 29.3% (51 patients) in the true hypertension group showed EBPR. The white-coat hypertension group showed the highest percentage of EBPR among all the groups.

In conclusion, the white-coat hypertension group and the true hypertension group showed significantly low HRR and higher prevalence of blunted HRR (Fig. 1).

DISCUSSION

The main finding of our study is that the parameter related with the nervous system responses to exercise such as HRR and EBPR are significantly different in the white-coat hypertension group from the normotensive group. These abnormal responses are similar to that of the true hypertension group. These results could repre-

Table 3. Parameters of the exercise test

	Control (n = 67)	White coat hypertension (n = 54)	True hypertension (n = 174)	ANOVA *P*
Exercise time, min	7.84 ± 2.32	7.83 ± 2.53	8.79 ± 2.02	0.066
METs	9.40 ± 2.59	9.55 ± 2.54	10.42 ± 2.41	0.004
Rest heart rate, /min	64.54 ± 13.34	63.43 ± 11.14	65.80 ± 9.59	0.059
Max heart rate, /,min	155.85 ± 26.38	151.29 ± 24.23	161.97 ± 19.61	0.072
HRR	69.85 ± 14.64	38.09 ± 28.75	28.76 ± 19.22	< 0.001
Blunted HRR, n(%)	0 (0)	18 (33.3)	48 (27.6)	< 0.001
Rest systolic BP, mmHg	116.49 ± 11.53	134.98 ± 16.42	141.59 ± 14.57	< 0.001
Rest diastolic BP, mmHg	70.67 ± 10.01	75.22 ± 13.28	82.01 ± 14.36	< 0.001
Max systolic BP, mmHg	165.92 ± 23.11	182.10 ± 22.02	188.66 ± 20.53	< 0.001
Max diastolic BP, mmHg	79.68 ± 12.92	83.41 ± 13.22	89.51 ± 14.09	0.010
EBPR, n(%)	3 (4.5)	17 (31.5)	51 (29.3)	< 0.001
QT	399.30 ± 37.27	404.36 ± 34.30	392.44 ± 32.3	0.920
QTc	428.72 ± 29.01	425.14 ± 24.34	425.93 ± 27.13	0.066

METs : metabolic equivalents, HRR : heart rate recovery, BP : blood pressure, EBPR : exagerated blood pressure response

sent another pathomechanism of white-coat hypertension.

The prognostic impact of white-coat hypertension is still a matter of debate and controversy.[19] Accumulating evidence focusing on the association of white-coat hypertension with subclinical target organ damage and, more importantly, incident cardiovascular disease suggests that the risk is intermediate between normotension and sustained hypertension. Pierdomenico et al.[20] stated that the white-coat hypertension group did not differ significantly from the normotensive group in terms of risk of a cardiovascular event, but because the white-coat hypertension group patients have much greater rate of antihypertensive treatment at follow up than the normotensive group, the results are not conclusive. The risk of cardiovascular event in white-coat hypertension was shown in a study by Verdecchia et al.[21] to be dependent on baseline value of BP on ABPM. In this study, data identified that the incidence of cardiovascular events tends to increase consistently above the daytime BP cut-off value of 130/80 mmHg. Similarly, as per the International Database of Home BP in relation to Cardiovascular Outcomes (IDHOCO), among the untreated subjects, cardiovascular risk was significantly higher in the white-coat hypertension group than in the normotensive group.[22] Furthermore, a meta-analysis of 14 studies with 29,100 participants performed by Briasoulis et al..[23] showed that the incidence of overall cardiovascular events was 6.0% in the white-coat hypertension subjects when compared to 4.0% in the normotensive subjects, thus meaning a 73% increased risk ($P < 0.001$). The risk for fatal cardiovascular events was increased even more, the incidence being 4.0% and 1.2% in the white-coat hypertension and normotensive groups, respectively.

There are many studies on the identification of the mechanism of white-coat hypertension, but these are not clear. The current study investigates whether individuals with white-coat hypertension have abnormal autonomic-cardiac regulation or impaired vascular function, similar to that observed in sustained or persistent hypertension. Sustained hypertension is associated with sympathetic predominance or diminished parasympathetic nervous system influences on heart rate, or both.[3, 24] Such "autonomic dysregulation" may be related to hypertension by increasing cardiac output, vascular resistance, and salt retention. Parasympathetic and sympathetic cardiac nervous system functions can be quantified noninvasively through spectral analysis of variability in heart rate.[25] Generally, an increase in HR during exercise occurs as a result of combined sympathetic activation and parasympathetic withdrawal.[26] In contrast, parasympathetic reactivation is the principal determinant of decreased HR during early recovery. Given the prognostic significance of diminished parasympathetic tone at rest, post-exercise HRR measurement is a noninvasive procedure that can be used to assess parasympathetic activation.[27] HRR is simple to calculate using the data obtained from standard exercise tests; moreover, because it does not require 24-h Holter monitoring or baro-reflex sensitivity testing, HRR may be valuable for assessing the risk in

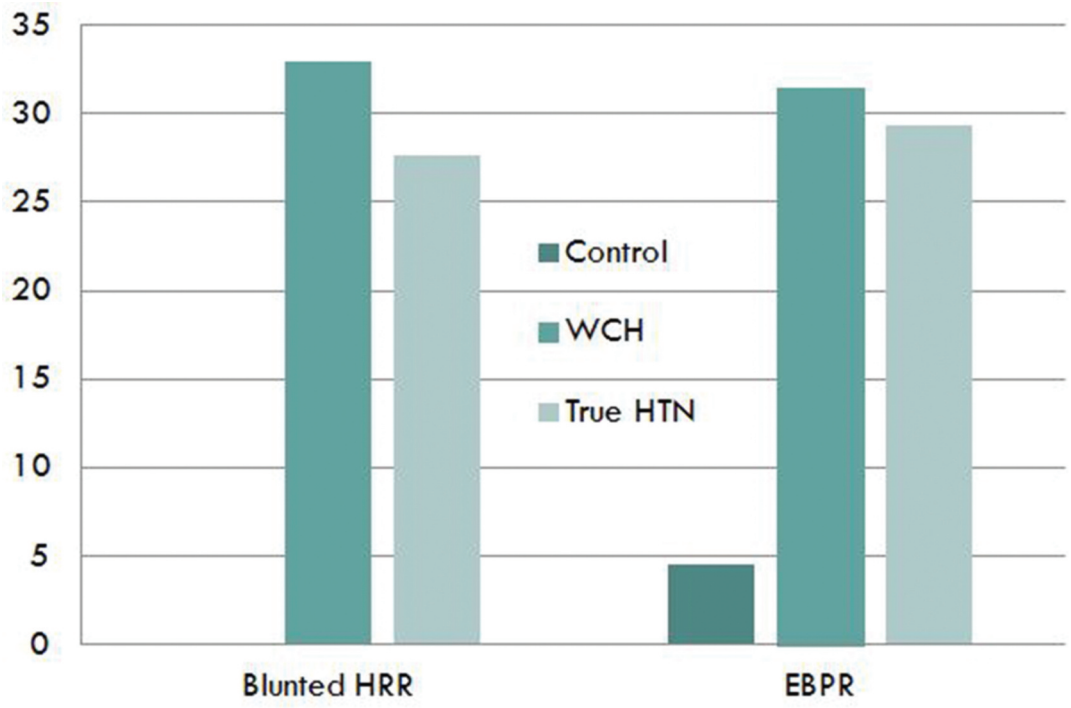

Fig. 1. The percentage of blunted heart rate recovery and exaggerated blood pressure response to exercise according to blood pressure pattern.

routine clinical practice, including exercise test.

EBPR is also related with the risk of future hypertension and cardiovascular mortality.[28] We observed that the white-coat hypertension group had a higher prevalence of EBPR, suggesting that white-coat hypertension might present due to the pathomechanism of impaired vascular response. A recent study by Androulakis el al. showed that the white-coat hypertension group presented with worse endothelial function, more pronounced arterial stiffness, and LVH.[29] BP reflects the changes in cardiac output during exercise or recovery that can lead to changes in SBP. Cardiovascular reactivity to both isometric and dynamic exercise has been shown to be one of the most important markers for predicting hypertension. Impaired vascular function, including increased arterial stiffness and abnormal endothelial function, is associated with increased exercise BP response.[30] Among the parameters of BP response, exercise BP response is an important marker of cardiovascular risk that is associated with cardiovascular mortality. In particular, EBPR is a significant predictor of cardiovascular events and for new onset of resting hypertension.

This study has several limitations. First, this is a retrospective study. Second, the cross-sectional study design eliminated our ability to determine causal relationships. Third, this study was performed at a single center, and relatively small number of subjects. So it is possible that biases existed with respect to patient referral and population sampling. In order to overcome this limitation, it is thought that additional large

randomised control studies will be needed. At last, our patients were treated by various medications that may have had some effect on BP. At base line, the normotensive group patients also were on RAS blockers, beta blockers, or Calcium channel blockers due to cardiovascular disease or renal disease. These medications might have had an impact on the BP variability and HRR.

Our findings suggest that autonomic dysregulation assessed by HRR and vascular dysfunction assessed by EBPR have a proportional relationship with white-coat hypertension like true hypertension. The presence of blunted HRR and EBPR showed significant association in the white-coat hypertension group when compared to the normotension group. This means that autonomic dysregulation and vascular dysfunction could be the pathomechanism of white-coat hypertension, and white-coat hypertension is a risk for a cardiovascular event. Based on these study findings, more attention is to be given to the role of white-coat hypertension in cardiovascular target organ damage, and more study in this area is warranted.

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Therapeutic Effects of Prolonged Release Melatonin (Circadin®) in Patients with Overactive Bladder and Chronic Insomnia in More Than 55 Years Old

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Objectives: Bladder storage symptoms including nocturia is the most common cause of sleep disturbance in all age groups. Sleep disturbance is also a main cause of nocturia so that sleep recovery can clinically improve nocturia. Melatonin has main action to induce sleep and additional effects of smooth muscle relaxation, free radical scavenging, anti-inflammation, et cetera. This study was evaluated the improvement of sleep quality after administrating prolonged-release melatonin in elderly patients with overactive bladder and chronic insomnia.

Methods: This clinical trial was performed with a randomized single open study. Thirty-seven patients with overactive bladder and chronic insomnia were initially enrolled in this study. After 4 or 12 weeks treating with 2 mg of prolonged-release melatonin, clinical outcomes were evaluated with OABSS, IPSS, PSQI and WHO 5 well-being index.

Results: Of the 37 patients, 34 (91.9%) were included in the ITT group and 26 (76.5%) in the PP group. In the primary outcome of PP group, significant improvements were observed in total OABSS and nocturia frequencies at 12 weeks, respectively. Secondary outcome measurement including in voiding, storage symptoms, and total IPSS scores showed the improvement at 4 and 12 weeks and in total and sleep quality PSQI scores at 12 weeks, and in quality of life scores of the WHO 5 well-being index at 12 weeks. Only one (3.8%) adverse event was observed.

Conclusions: These results suggest clearly that prolonged-release melatonin in elderly patients with overactive bladder and chronic insomnia has the potential to control concomitant voiding and sleep difficulty.

Key Words: Insomnia, Melatonin, Nocturia, Overactive Bladder

The prevalence of various urological conditions in men more than 55 years old are increasing similar to the incidence of sleep disorder, which included nocturia, low urinary tract symptoms (LUTS), overactive bladder (OAB), benign prostatic hyperplasia (BPH), bladder & prostate cancer, cystitis, prostatitis, neurogenic bladder, urethral stricture, nocturnal polyuria, and testosterone deficiency syndrome (TDS).[1-3] Of them, nocturia is thought to be primarily affected by these pathological conditions and to influence significantly quality of life as a most common cause of sleep disturbance in all age groups.[4,5]

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Normal sleep requirement is essential for body homeostasis which normal duration of sleep is 16-18 hours in neonate, 10 hours in infant & early child, 8 hours in adolescence and 7-8 hours in adult. Prevalence of insomnia is sleep disorder in 40 to 70% as well as primary insomnia in 10 to 24% of elderly.[1]

Melatonin is as a major regulatory hormone in circadian rhythm for normal night sleep which is present during normal sleep hours. Melatonin secretion and prevalence of insomnia, BPH/LUTS and TDS are showed with significant correlation according to age. Prolonged-release melatonin (Circadin®, Kuhnil Pharm, Seoul, Korea) is once a daily oral agent on bedtime that is particularly effective for patient with primary insomnia.[6] Objective of this study is to evaluate the clinical efficacy of prolonged release melatonin in patients with overactive bladder and chronic insomnia in elderly male.

MATERIALS AND METHODS

Study design

This clinical trial was performed with a pilot randomized non-placebo controlled single center registry-based prospective clinical trial as investigator intended post-marketing testing in accordance with the Good Clinical Practices standards and in conformity with the ethical principles set out in the Declaration of Helsinki. Demographic characteristic, medical history, presenting symptoms, and variety of treatment outcome data were collected by trained nurses using a standardized case report form at each site.

Subject screening and clinical outcomes during 12 weeks treating with Circadin® were evaluated with overactive bladder symptom score (OABSS), international prostate symptom score (IPSS), Pittsburgh sleep index (PSQI) and WHO 5 well-being index. For the assessment of sleep quality, a specialist in sleep disorders was recommended the PSQI which was a self-report questionnaire.[7-11] Screening for subject suitability was performed with OABSS, IPSS and PSQI with pretrial wash-out at least 4 weeks before administration of the investigational product on visit 1. At visit 2, 3 and 4, OABSS, IPSS and PSQI were reviewed with the subject's diary to be conducted at baseline and after 4 and 12 weeks of treatment. At visit 2 and 4, WHO 5 well-being index were also evaluated with checking physical examination, vital signs, laboratory test and echocardiography. At visit 4 after medication, adverse event was evaluated (Fig. 1). Randomization numbers were given to patients who were judged to be suitable for the clinical trial.

Subjects

The subject criteria to be enrolled in the screening test were OABSS scale 2 $\geq$ 2, OABSS scale 3 $\geq$ 2, and PSQI $\geq$ 5. Inclusion criteria in this study are adult male more than 55 years, history of OAB recent 3 months or more, total score 3 < and Q2 2 < on overactive bladder symptom score, patient with chronic insomnia and completed patient informed consent prior to clinical trial. The exclusion criteria are as follows; clin-

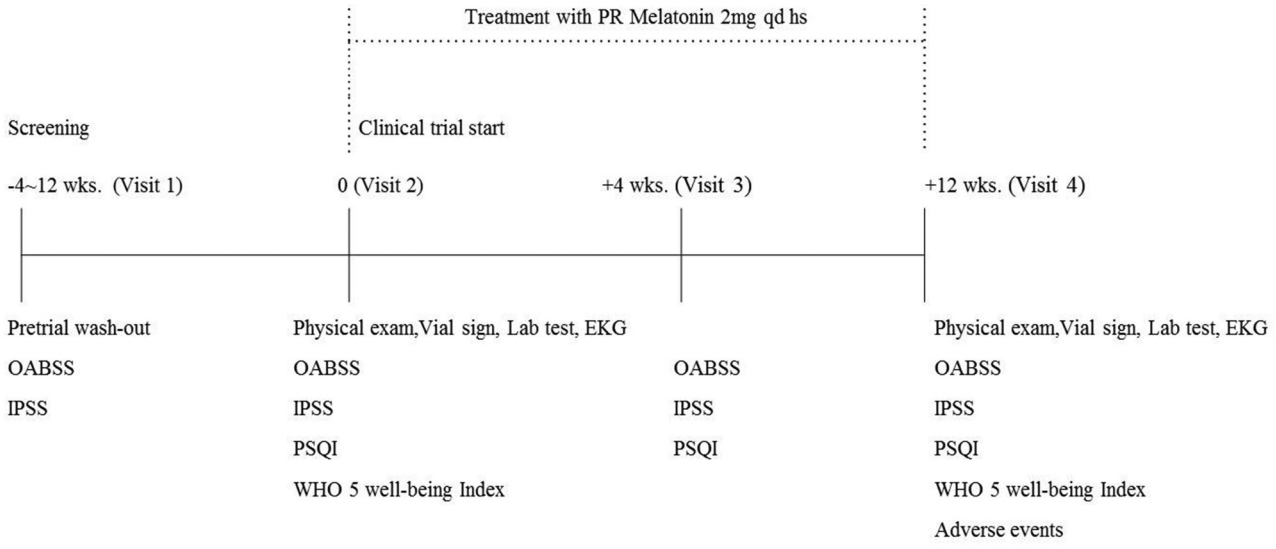

Fig. 1. Study design and flow of Circadin® clinical trial study

ically significant bladder outlet obstruction, patient with overactive bladder due to neuropathic causes, patient with bladder stone, interstitial cystitis, active urinary tract infection, patient with previous or current pelvic organ cancer, patient with secondary insomnia due to obstructive sleep apnea, chronic pain or restless legs syndrome, case with irregular sleep pattern, shift-worker, case to have contraindicated medication during clinical trial, patient with clinically serious liver or kidney disease by investigator decision making, participant of other clinical trial, and case not to visit according to time line of clinical trial. Concomitant medications to be prohibited are alpha blocker, 5-alpha reductase inhibitor, anticholinergic agents, antimuscarinic agents, herbal medications for voiding difficulty or overactive bladder, cimetidine, adrenalin agonist and antagonist, opioid agonist and antagonist, antidepressant, prostaglandin inhibitor, benzodiazepine and non-benzodiazepine hypnotics, imipramine, thioridazine, methoxsalen, fluvoxamine, CYP3A4 inhibitors including ritonavir, saquinavir, ketoconazole, itraconazole, erythromycin, Clarithromycin, grapefruit juice, voriconazole, indinavir and nelfinavir, CYP1A2 inhibitor including quinolone, CYP1A2 inducing agents including rifampicin and carbamazepine.

Ethics statement

The study protocol was approved by the Institutional Review Board at Pusan National Hospital (PNUH IRB No. D-1704-002-064 (Apr. 27, 2017) as well as Korea Food and Drug Administration (KFDA) Approval No. 31223 (March 08, 2017). Informed consent was obtained by all subjects when they were enrolled.

Investigational drug

Circadin® (Kuhnil Pharm, Seoul, Korea), originally developed by H. Lundbeck A/S (Copen-

hagen, Denmark) , is a prolonged-release medicine of 8-10 hours to be possible full night melatonin coverage.[6] Dosage and administration are 2 mg of prolonged-release melatonin on 1-2 hours before everyday bedtime.

Efficacy assessments

Primary end point for efficacy outcome assessments were changes of OABSS with total score and Q2 (nocturia frequency) score from baseline to 4 and 12 weeks. Secondary end points included changes of IPSS with total score, voiding symptom score and storage symptom score, PSQI total score, PSQI Q9 score from baseline to 4 and 12 weeks, changes of WHO 5 well-being Index (1998 version) for evaluating quality of life, total score, on baseline and after 12 weeks of treatment.

Safety evaluation

Safety evaluation at weeks 4 and 12 included physical examination, vital signs, laboratory test, echocardiography and findings. Adverse events were assessed for patients who took at least 1 dose of Circadin®. Adverse events reported in response to general and non-specific inquiry survey by the researcher or self-reported by the patient were described with severity at each visit according to the World Health Organization (WHO) Adverse Reactions Terminology (WHOART) system organ classes. Adverse drug reactions related to the investigational product were compared using the same method.

Statistical analysis

The intention-to-treat (ITT) analysis was conducted for all randomized subjects to analysis of all subjects initially enrolled in a clinical trial. The per-protocol (PP) population was defined as those subjects in the ITT population who had completed the visit and for whom there were no serious protocol deviations. In this study of PP group, the treatment efficacy was determined by comparing primary and secondary outcome measures at the end of each treatment.

According to the standards of a per-protocol analysis, sample size was calculated with 37 participants by G Power 3.1, at least 34 participants to be recruited plus 10% unfaithful responders. Statistical analysis of data from inquiry survey was performed by paired *t*-test. Changes from baseline in continuous safety variables including vital signs, laboratory analysis, echocardiographic finding and were evaluated by ANOVA. Efficacy measured in ITT and PP groups included the mean ± SD changes at 4 and 12 weeks from baseline. Statistical significance was accepted for P values of < 0.05.

RESULTS

Demographics and Baseline Characteristics

The patients in this study were recruited consecutively and evaluated prospectively for 1 year between July 2017 and August 2018. Initially, eligible patients had minimal 4 weeks and maximally up to 12 weeks treatment-free run-in period and then were checked for adequacy for inclusion. Thirty seven patients were screened in

this study. A total of 34 patients were enrolled in the ITT group. Overall, 26 patients (76.5%) of these were completed the study in the PP group. The demographics and baseline characteristics of the volunteers in the ITT and PP groups are shown Table 1. Data from two groups were used to analyze efficacy parameters. Mean patient age in the ITT and PP groups were 69.5 ± 7.7 and 69.8 ± 7.7 years, respectively. At baseline, no clinically or statistically meaningful difference was found between the two groups with respect to demographic or clinical variables.

Table 1. Clinical profile of patients

	ITT group (%)	PP group (%)
Age (decades)		
'50	7 (20.6)	5 (19.2)
'60	6 (17.6)	5 (19.2)
'70	19 (55.9)	14 (53,8)
'80	2 (5.9)	2 (7.7)
Total	34(100.0)	26(100.0)
Mean ± 2SD (years)	69.5 ± 7.7	69.8 ± 7.5
Body Weight (kg)		
40-49	1 (2.9)	1 (3.8)
50-59	3 (8.8)	3 (11.5)
60-69	14 (41.2)	9 (34.6)
70-79	16 (47.1)	13 (50.0)
Total	34(100.0)	26(100.0)
Mean ± 2SD	67.8 ± 6.6	67.2 ± 7.0
Height (cm)		
160-170	21 (61.8)	16 (61.5%)
>170	13 (38.2)	10 (38.5%)
Total	34(100.0)	26(100.0)
Mean ± 2SD	168.3 ± 4.2	168.2 ± 3.9
Duration of chronic insomnia		
< 6 months	1 (2.9)	1 (3.8)
6 months – 3 years	26 (76.5)	19 (73.1)
3 – 5 years	6 (17.6)	5 (19.2)
5 years <	1 (2.9)	1 (3.8)
Total	34(100.0)	26(100.0)
Mean ± 2SD (months)	23.0 ± 13.0	23.0 ± 14.1
Overactive Bladder symptom score		
Mild	11 (32.	7 (26.9)
Moderate	20 (58.8)	17 (65.4)
Sever	3 (8.8)	2 (7.7)
Total	34(100.0)	26(100.0)
Mean ± 2SD (months)	7.56 ± 3.174	7.85 ± 3.081
International Prostate Symptom Score		
Mild	-	-
Moderate	13 (38.2)	10 (38.5)
Sever	3 (8.8)	16 (61.5)
Total	34(100.0)	26(100.0)
Mean ± 2SD (months)	22.94 ± 7.447	22.96 ± 7.922

Values are presented as mean ± standard deviation.

Efficacy outcomes

Data on the effectiveness of this clinical trial were conducted by the ITT group which enrolled efficacy assessment after receiving at least one clinical treatment. The PP group followed visitors for 12 weeks without major violations among the ITT group, the targeted 26 patients with a compliance rate of 76.5% was included in the PP group. Analysis of primary and secondary efficacy endpoints was only measured on the PP group.

Primary efficacy outcome

From baseline to follow-up, mean total OABSS scores reduced in the PP group from 7.85 ± 3.08 (baseline) to 5.69 ± 3.08 (12 weeks, $P < 0.001$) (Fig. 2A). The PP group also showed the reduction of nocturia frequency from 2.88 ± 0.33 (baseline) to 2.12 ± 0.27 (12 weeks, $P < 0.001$) (Fig. 2B).

Secondary efficacy outcome variables

Secondary endpoints were evaluated the change of total IPSS, voiding, and storage symptom in the PP group. From baseline to follow-up, mean total IPSS scores were reduced from 22.96 ± 7.92 (baseline) to 18.73 ± 5.23 (4 weeks, $P < 0.01$), 16.27 ± 5.39 (12 weeks, $P < 0.001$) (Fig. 3A). Overall voiding symptom scores was decreased from 12.58 ± 4.67 (baseline) to 10.69 ± 4.22 (4 weeks, $P < 0.001$), and 9.96 ± 4.12 (12 weeks, $P < 0.001$) (Fig. 3B). Storage symptom were also reduced from 9.5 ± 3.09 (baseline) to 8.2 ± 2.63 (4 weeks, $P < 0.001$), 6.31 ± 2.56 (12 weeks, $P < 0.001$) (Fig. 3C).

Study participants were evaluated by using total PSQI and sleep quality to evaluate of the melatonin administration in the PP group. From baseline to follow-up, mean total PSQI scores were reduced from 8.42 ± 4.70 (baseline) to 7.0 ± 4.03 (12 weeks, $P < 0.001$) (Fig. 3D). Poor sleep qual-

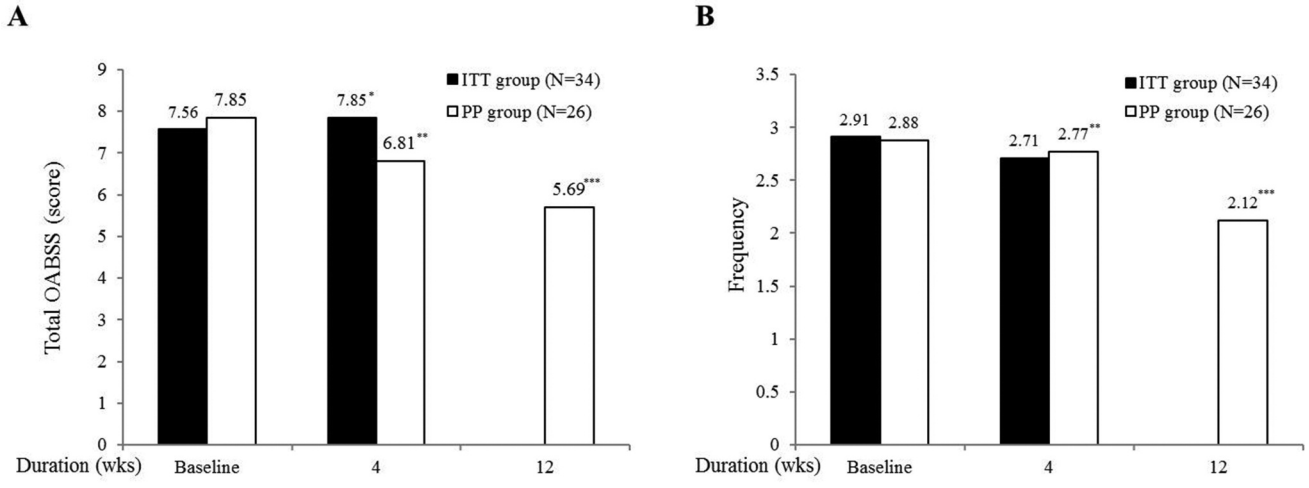

Fig. 2. Changes of total score of overactive bladder symptom score (OABSS) and nocturia frequency
(A) Total OABSS, (B) The nocturia frequency, Values are presented as mean ± standard deviation. *$P > 0.05$ vs baseline, ** $P < 0.01$ vs baseline, *** $P < 0.001$ vs baseline

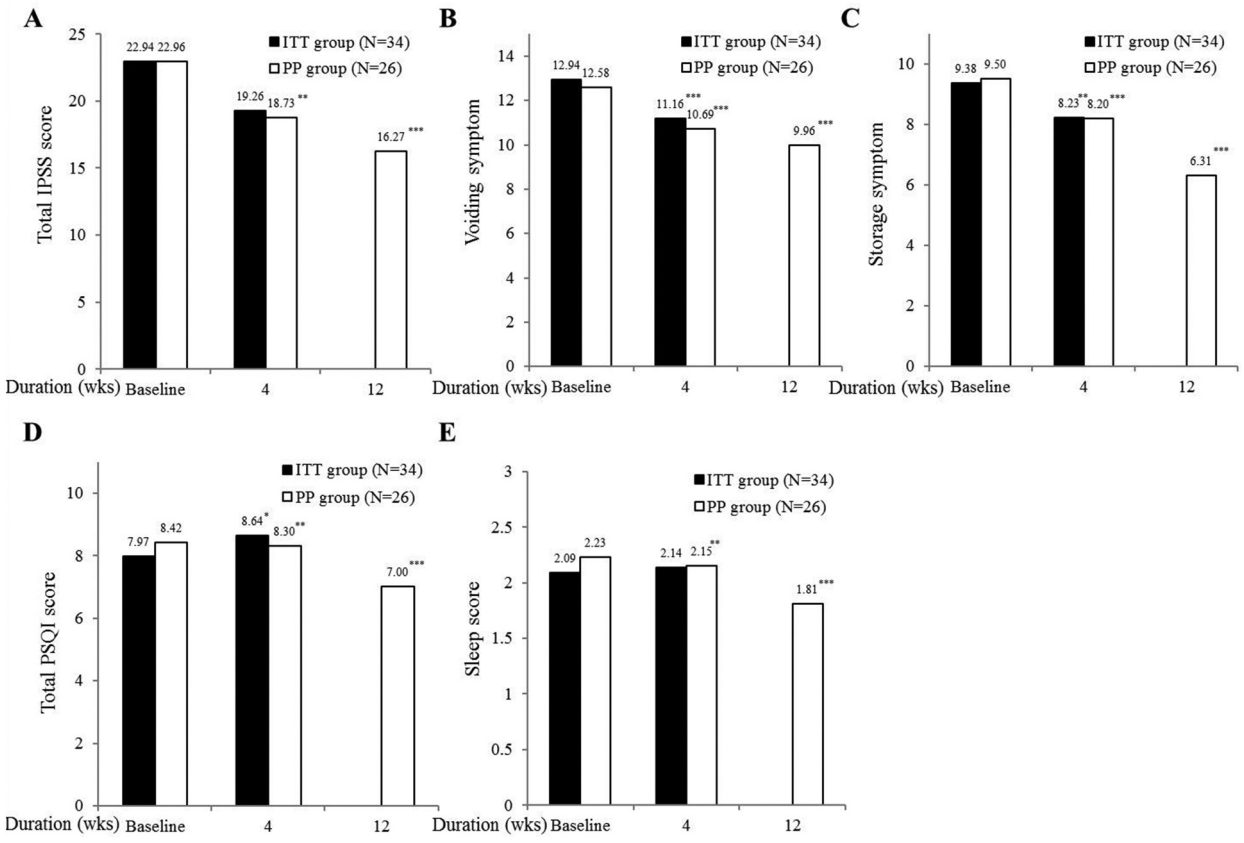

Fig. 3. Change in mean total IPSS, voiding and storage symptom, total PSQI, and sleep quality
(A) Total IPSS Score, (B) Voiding Symptoms, (C) Storage Symptoms, (D) Total PSQI Score, (E) Quality of Sleep * *P* > 0.05 vs baseline, ** *P* < 0.01 vs baseline, * *P* < 0.001 vs baseline. Values are presented as mean ± standard deviation.**

ity were also reduced from 2.23 ± 0.59 (baseline) to 1.81 ± 0.57 (12 weeks, $P < 0.001$) (Fig. 3E).

PP analysis including only patients with treatment compliance also showed improvement of WHO 5 quality of life scores from 10.15 ± 6.44 (baseline) to 13.54 ± 5.24 (12 weeks, $P < 0.001$) (Fig. 4).

Safety and tolerability

In total, 34 subjects who took at least one dose were included in the safety analysis of Circadin®. Patients with medication were generally well tolerated with no adverse event (AE) during the study or follow-up period. Adverse event was observed only one case (3.8%) with mild degree eyelid edema. This event was recovered spontaneously within 1 week. No clinically significant changes in laboratory tests, electrocardiogram, or blood pressure were observed in treatment group.

DISCUSSION

In elderly, age-related changes in sleep depth and continuity affect normal circadian rhythm,

and the normal circadian pattern of micturition as well. It is well known that the prevalence of nocturia increases with age which is associated with poor quality of life as well as self-reported insomnia.[12] Nocturia is the most common bladder storage symptom in the general population.[13] Three-quarters of participants in a survey of 8937 non-institutionalized individuals aged 18 years or over living in Texas, New York and California states residents of US cited the need to go to the bathroom as the most frequent reason for nocturnal awakenings. Indeed, going to the toilet was the primary reason for night-time awakening across all age groups, and the proportion affected increased with age: 39.9% in those aged 18–44 years to 77.1% in those aged 65 or above.[5] Therefore, primary sleep hygiene tip is to improve sleep and nocturnal urinary frequency together in patients with LUTS/OAB.

Nocturnal voids are regulated by circadian biological rhythms that include decreased nocturnal urine production through urine concentration via water reabsorption or through sodium retention, plasma renin angiotensin-aldosterone system, vasopressin with a peak diurnal rhythm during the night time hours, and atrial natriuretic peptide with important role in sodium excretion at night.[14,15]

Currently, noctuira is treated successfully with various options including alpha blocker for prostate diseases, anticholinergics for bladder storage function and desmopressin for replacing antidiuretic hormone.[16-18] Nevertheless, hypnotics and desmopressin increase the potential risk of dependency and hyponatremia, especially in the elderly. Therefore the new option to control nocturnal frequency and insomnia together is still required in the clinical field.

Melatonin is a pineal gland hormone to exhibit a circadian rhythm, which shows low level during on daytime due to inhibition of its production by light and high level during night after onset darkness. Generally, it is considered melatonin is able to stabilize circadian rhythms, to reinforce them, and to maintain their phase-relationships, and thus, melatonin acts as an endogenous synchronizer of circadian rhythms and sleep is normally initiated when blood melatonin levels increase.[19,20]

Melatonin has been found to have several physiologic effects on bladders in animal models, which include pelvic smooth muscle and bladder detrusor muscle tone adjustments (due to decreasing sympathetic tone), vascular tone adjustment, anti-oxidative effects (due to free radical scavenging), and anti-inflammatory effects, and to improve sleep quality.[21-28] Matsuta et al. reported that exogenous melatonin increases bladder capacity vias γ-aminobutyric acidA receptor in the GABAergic system.[29] These observations suggest melatonin could be beneficial for treating nocturia due to its effects on the central nervous system. Obayashi et al reported melatonin secretion was significantly and inversely associated with nocturia in a cohort study of an elderly population.[30] This study was the first clinical study to evaluate the improvement of urination functions such as nocturia based on the anticholinergic action of melatonin on the bladder that was confirmed in animal experiments.

These physiologic actions of melatonin are expected to enable normal voiding volumes and urinary frequencies by increasing functional bladder capacity, inter-contraction intervals, and threshold pressure and decreasing basal pressure on bladder (Fig. 5).

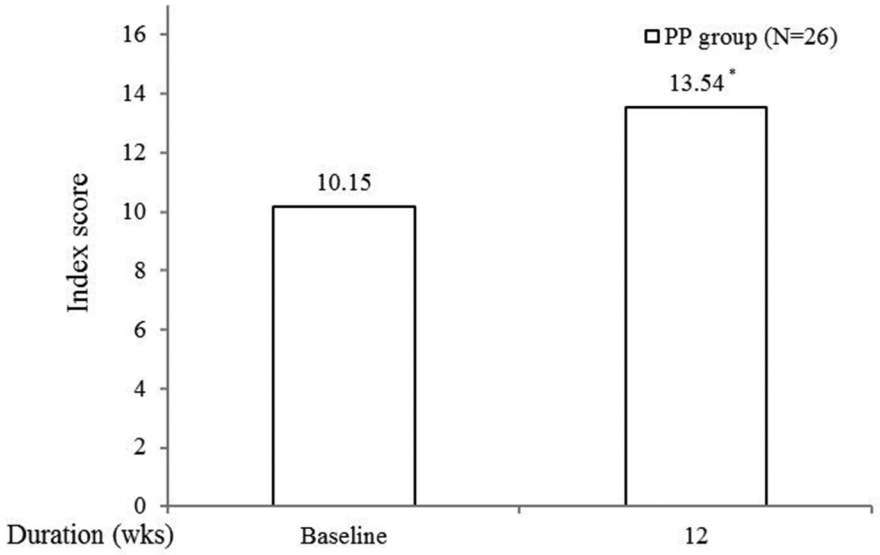

Fig. 4. Changes of quality of life by WHO 5 well-being index
Values are presented as mean ± standard deviation.* *P* < 0.001 vs baseline

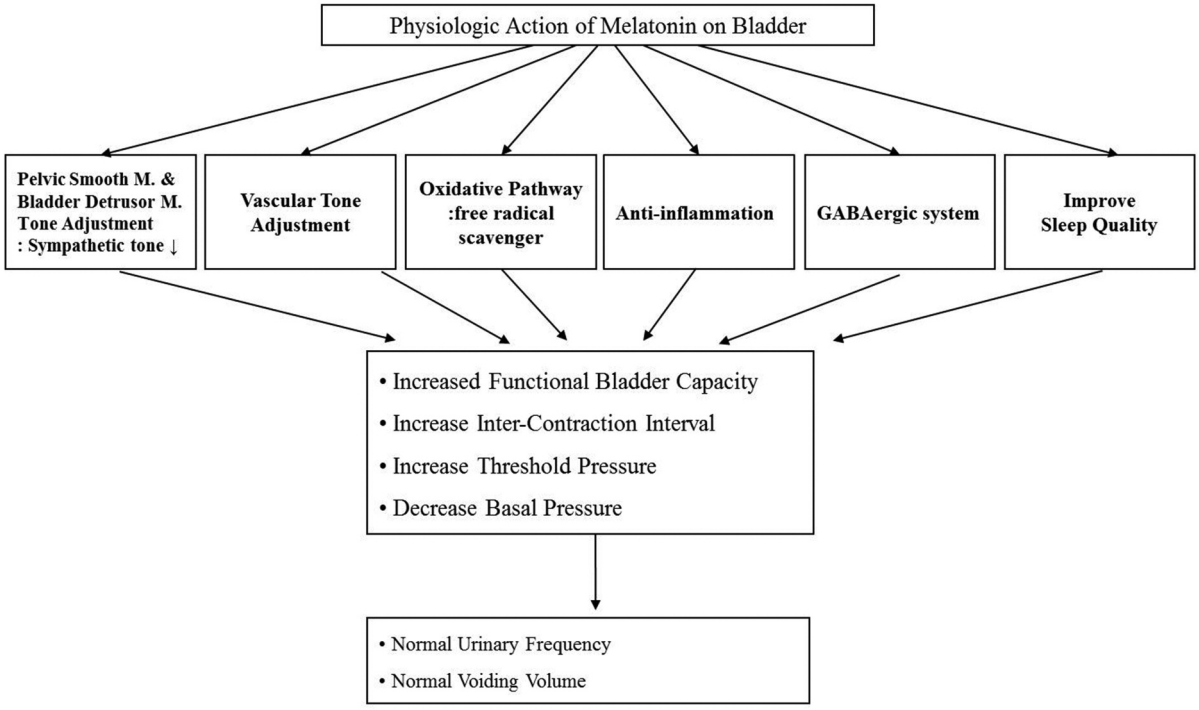

Fig. 5. Physiologic actions of melatonin on bladder

We hypothesized that a reduction in symptoms of overactive bladder symptoms and improved sleep quality might be directly or indirectly achieved by administering melatonin, melatonin plus an alpha blocker, an anticholinergic agent, or an antidiuretic hormone. Circadin® (Kuhnil Pharm, Seoul, Korea) releases melatonin over 8-10 hours, and thus, should provide full night coverage, whereas nutritional supplements release it over 2-3 hours.[6] The present clinical trial is meaningful because no previously study has investigated the role of melatonin on human bladder function from a clinical perspective. Our results show that 2 mg of prolonged release melatonin daily significantly improved almost all measures and was well tolerated with no adverse events leading to discontinuation. The reasons for drop-out of some study subjects (23.5%) were withdrawal of consent, non-compliance, and inclusion and exclusion violations during screening or the first 4 weeks of the clinical trial. This pilot study was performed as a global first clinical trial to investigate the effects of melatonin on bladder function. Although the findings of this study suggest that prolonged release melatonin has acceptable efficacy and safety, some limitations should be noted. First, the study design was established as non-placebo controlled, open label, single center study. Second, the present study was designed to have a relatively short period of treatment of 12 weeks. Long-term data are therefore needed as in other clinical trial studies. Third, the efficacy was not assessed according to the severity of chronic insomnia and overactive bladder. And comparison data was not shown with other treatment options. Future studies of prolonged release melatonin should include patients not to response to treatment such as failed those with current therapeutic modalities.

These results was shown clearly that the clinical use of prolonged-release melatonin (Circadin®) in elderly patients with overactive bladder and chronic insomnia has the potential to control simultaneously voiding symptom and poor sleep quality even though it was not a placebo controlled long-term study. Based on these results, we suggest that prolonged release melatonin is sleep health enhancer with anticholinergic effect which can be used as primary prescription in patients with LUTS/OAB and sleep disorder together. Additionally sleep pattern should be evaluated on primary care of patients with urological disorders with BPH, LUTS or OAB.

CONFLICT OF INTEREST

The authors reported no conflicts of interest to declare in this article.

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Differences in Endoscopic Findings of Primary and Secondary Gastric Lymphoma

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Objectives: Since endoscopic findings of primary gastric lymphoma are ambiguous and diverse, it is not easy to distinguish them from gastric adenocarcinoma or secondary gastric lymphoma. The aim of this study was to investigate the difference in clinical and endoscopic features between primary gastric lymphoma and gastric involvement of lymphoma.

Methods: Forty-eight patients were enrolled in this retrospective study between June 2008 and February 2017. The patients were divided into primary gastric lymphoma group (primary group, n = 18) and gastric involvement group (secondary group, n = 30) based on whether or not they carried gastric lesions alone. Patients' clinical characteristics, endoscopic findings and pathologic data were retrospectively reviewed based on electronic medical records.

Results: The mean age of patients was 63.3 ± 13.1 years and 29 patients were female (60.4%). Diffuse large B-cell lymphoma pathology (81.3%), gastric body involvement (47.9%) and ulceroinfiltrative morphology on endoscopy (43.8%) were common features. Regardless of the two groups, the initial endoscopic diagnosis was considered as lymphoma only in 41.7%. Compared with the primary group, fundus ($P = 0.035$) and regional lymph node ($P < 0.001$) were significantly associated with the secondary group. However, there was no significant difference in endoscopic findings including location, size, number, and morphology of lesion.

Conclusions: Endoscopic diagnosis of gastric lymphoma is a challenge. There is no difference in endoscopic findings between the primary and secondary groups even when confirmed separately. However, when the lesion is present in the fundus, we keep in mind the possibility of secondary gastric lymphoma.

Key Words: Endoscopy, Lymphoma, Stomach neoplasm

The gastrointestinal tract is the most common site of extra-nodal involvement in lymphoma. The stomach is the most frequent site of gastrointestinal involvement, followed by the small intestine and ileocecal area.[1] Among the gastric lymphomas, primary gastric lymphoma (PGL) originates in the stomach, with or without perigastric and/or abdominal lymph node involvement. PGL is an uncommon tumor, accounting for less than 15% of gastric malignancies and about 2% of all lymphomas,[1-3] and diffuse large B-cell lymphoma (DLBCL) is the most common type of primary gastric lymphoma.[1] Due to the ambiguity and diversity of endoscopic findings, gastric lymphoma cannot be easily distinguished from gastric adenocarcinoma.

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Although several recent studies have addressed the clinical and endoscopic features of gastric lymphoma, the diagnosis of gastric lymphoma remains a challenge, especially in the early stage of the disease, which decreases the likelihood of successful management.[4,5] As it is difficult to distinguish gastric cancer from gastric lymphoma, it is also difficult to distinguish primary gastric lymphoma from gastric involvement of systemic lymphoma.

This study investigated the possible strategies for endoscopists to diagnose primary lymphoma more efficiently by addressing the diagnostic challenges. Therefore, the aim of this study was to investigate the difference in clinical and endoscopic features between primary gastric lymphoma and gastric involvement of lymphoma. We will describe three pair of cases which was difficult to tell which one is primary or secondary lymphoma case.

MATERIALS AND METHODS

Patient population

This study was a retrospective study conducted at the single tertiary center and involved lymphomas confirmed by endoscopic biopsy from June 2008 to February 2017. Forty-eight patients were enrolled in this study. The enrolled patients were divided into a primary gastric lymphoma group (primary group, n = 17) and a gastric involvement group (secondary group, n = 31) based on the presence of only gastric lesion (Fig. 1). Patients' clinical characteristics, endoscopic findings and pathologic data were retrospectively reviewed using electronic medical records. The study was approved by our Institutional Review Board. All procedures were in accordance with the ethical standards of the Declaration of Helsinki. (IRB No.: KUGH 2019-10-021)

Primary and secondary gastric lymphoma

Primary gastric lymphoma was defined by the gastric location of the main lesion, with or without perigastric and/or abdominal lymph node involvement.[6] In the present study, the primary

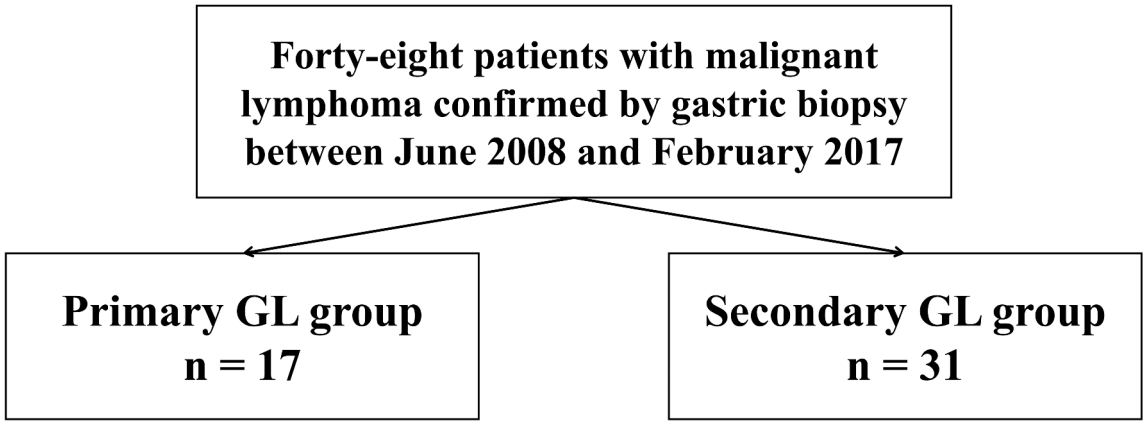

Fig. 1. Flow chart of patients (Abbreviation. GL, gastric lymphoma).

gastric lymphoma was defined based on the following Dawson's criteria: 1) absence of peripheral lymphadenopathy at the time of presentation, 2) lack of enlarged mediastinal lymph nodes, 3) normal total and differential white blood cell counts, 4) predominance of gastric lesions at the time of laparotomy with only lymph nodes apparently affected in the immediate vicinity, 5) no lymphomatous involvement of liver and spleen.[7] On the other hand, cases of secondary lymphoma were defined by distant lymph node involvement or involvement of other organs, deviating from the definition of primary lymphoma.

At time of initial diagnosis of gastric lymphoma, biopsy specimens from stomach, lymph node, or bone marrow were processed routinely with preparation of hematoxylin-eosin–stained sections. If initial hematoxylin-eosin stained slide showed suspicious lymphoma, fixed, paraffin-embedded tissue specimens were assessed immunohistochemically by pathologists using antibodies specific for CD3, CD5, CD10, CD20, cyclin D1, bcl-2, bcl-6, Cytokeratin cocktail, EMA and MUM-1. Some specimens also assessed for B-cell monoclonality using Immunoglobulin Heavy Chain (Ig H) gene rearrangement.

Endoscopic classification

Endoscopic findings listed the most frequently mentioned items in the literature previously associated with gastric lymphoma. The most redundant and frequently mentioned findings were classified into 8 groups as follows: nodular, polypoid, ulcerofungating, ulceroinfiltrative, erosive, diffuse infiltrating, thickened fold-like, and mixed types.[4,8-10] Representative cases under each classification are illustrated in Fig. 2.

Statistical analysis

The categorical variables were compared using Pearson's chi-square, Fischer's Exact test or linear by linear association, whereas the continuous variables were compared using Student's t-test where appropriate. Statistical analysis was performed using IBM SPSS Statistics version 24.0 (IBM Co., Armonk, NY, USA) with a statistical significance level set at $P < 0.05$.

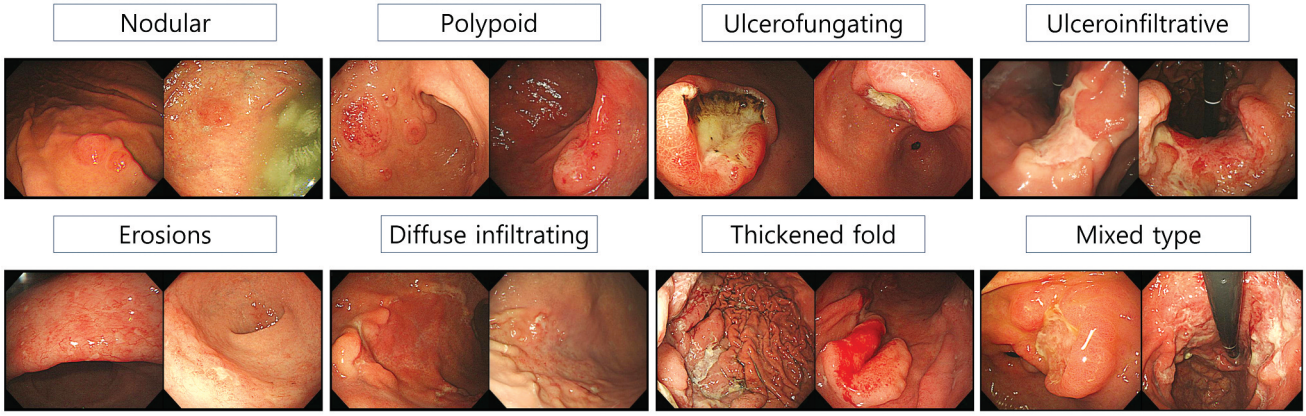

Fig. 2. Endoscopic classification for gastric lymphomas

RESULTS

1. Baseline characteristics

The mean age of the patients was 63.3 ± 13.1 years and 29 patients were female (60.4%). There was no clinical difference between patients with primary and secondary lymphomas, except for the involvement of the fundus (P = 0.035), when the invasion site of the gastric lesion was divided into the antrum, body, and fundus, respectively (Table 1). Only 41.7% (20/48) cases considered lymphoma in the first endoscopic diagnosis, including both primary and secondary lymphoma.

2. Clinicopathologic findings

DLBLC was the most frequently diagnosed pathology (81.3%) based on all the histological findings in this study, similarly to previous studies. Approximately 56% of the invasion depth in the stomach was confined to the mucosa and submucosal layer, and there was no difference in primary and secondary lesions (Table 2). Involvement of lymph node in the upper gastroin-

Table 1. Baseline and endoscopic characteristics of patients diagnosed with primary and secondary gastric lymphoma

Variables	Primary GL (n = 17)	Secondary GL (n = 31)	*P*-value
Age (years)	65.52 ± 12.04	62.19 ± 13.86	0.933
Sex			0.541
Male	8 (47.1%)	11 (35.5%)	
Female	9 (52.9%)	20 (64.5%)	
Involvement site			
Antrum (+)	8 (47.1%)	14 (45.2%)	1.000
Antrum (-)	9 (52.9%)	17 (54.8%)	
Body (+)	15 (88.2%)	21 (67.7%)	0.169
Body (-)	2 (11.8%)	10 (32.3%)	
Fundus (+)	1 (5.9%)	11 (35.5%)	0.035
Fundus (-)	16 (94.1%)	20 (64.5%)	
More than 2 sites (+)	6 (35.3%)	11 (35.5%)	1.000
(-)	11 (64.7%)	20 (64.5%)	
Growth pattern			0.131
Unifocal	11 (64.7%)	12 (38.7%)	
Multifocal	6 (35.3%)	19 (61.3%)	
Initial endoscopic impression			0.429
Lymphoma	7 (41.2%)	13 (41.9%)	
EGC	3 (17.6%)	1 (3.2%)	
AGC	4 (23.5%)	8 (25.8%)	
MALT lymphoma	2 (11.8%)	2 (6.5%)	
Others	1 (5.9%)	7 (22.6%)	

Abbreviation. GL, gastric lymphoma; EGC, early gastric cancer; AGC, advanced gastric cancer; MALT, mucosa-associated lymphoid tissue

Data are expressed as number (%). *P* values were calculated with the use of Pearson's chi-square test, Fisher's exact test or linear by linear association except age (Student's T-test).

Table 2. Clinical and pathologic characteristics of patients according to the primary and secondary gastric lymphoma

Variables	Primary GL (n = 17)	Secondary GL (n = 31)	*P*-value
Pathology			0.111
DLBCL	16 (94.1)	23 (74.2)	
Mantle cell lymphoma	1 (5.9)	7 (22.6)	
Burkitt lymphoma	0 (0.0)	1 (3.2)	
Depth of invasion			0.544
M & SM	11 (64.7)	16 (51.6)	
Beyond SM	6 (35.3)	15 (48.4)	
Regional lymph node			<0.001
Negative	12 (70.6)	3 (9.7)	
Positive	5 (29.4)	28 (90.3)	
Bone marrow involvement			0.155
Negative	14 (100.0)	24 (80.0)	
Positive	0 (0.0)	6 (20.0)	
Not evaluated	3	1	
Helicobacter pylori status			0.080
Negative	2 (25.0)	9 (69.2)	
Positive	6 (75.0)	4 (30.8)	
Not evaluated	9	18	

Abbreviation. GL, gastric lymphoma; DLBCL, diffuse large B-cell lymphoma; M mucosa; SM, submucosa
Data are expressed as number (%). *P* values were calculated with the use of Pearson's chi-square test, Fisher's exact test or linear by linear association.

testinal tract was significantly higher in secondary than in 29.4% of primary and 90.3% of secondary cases. Bone marrow test was performed in about 90% of cases, whereas the invasion was more frequent in secondary cases than in primary lymphoma. In 43% of cases tested for *Helicobacter pylori*, no difference in the pathology of primary and secondary lymphomas was detected.

3. Endoscopic findings

No statistically significant differences were detected in the eight different types of primary and secondary gastric lymphoma based on endoscopic findings. However, the ulceroinfiltrative type, similar to a Borrmann type 3 stomach cancer was the most common, followed by the mixed type in both groups (Table 3).

4. Primary and secondary lymphoma cases difficult to distinguish

The three representative cases included in this study are as follows. First, both cases were classified as polyoid lesions, with histological findings of diffuse large B-cell lymphoma. However, a case was classified as secondary lymphoma with ileal lymph node involvement. Both cases were difficult to differentiate because of similar endoscopic features (Fig. 3). Second,

both cases showed ulcercofiltrative lesion, although primary gastric lymphoma was confirmed as mantle-cell lymphoma and secondary lymphoma showed diffuse large B-cell lymphoma histology. In secondary gastric lymphoma, positron emission tomography-computed tomography (PET-CT) showed invasion of multiple whole bones, bone marrow, left

Table 3. Endoscopic findings of gastric lymphomas

Variables	Primary GL (n = 17)	Secondary GL (n = 31)	*P*-value
Endoscopic findings			0.941
Nodular	0 (0.0)	2 (6.5)	
Polypoid	1 (5.9)	3 (9.7)	
Ulcerofungating	0 (0)	3 (9.7)	
Ulceroinfiltrative	11 (64.7)	10 (32.3)	
Erosions	1 (5.9)	3 (9.7)	
Diffuse infiltrating	1 (5.9)	1 (3.2)	
Thickened fold	1 (5.9)	2 (6.5)	
Mixed type	2 (11.8)	7 (22.6)	

Abbreviation. GL, gastric lymphoma
Data are expressed as number (%). *P* value was calculated by linear by linear association.

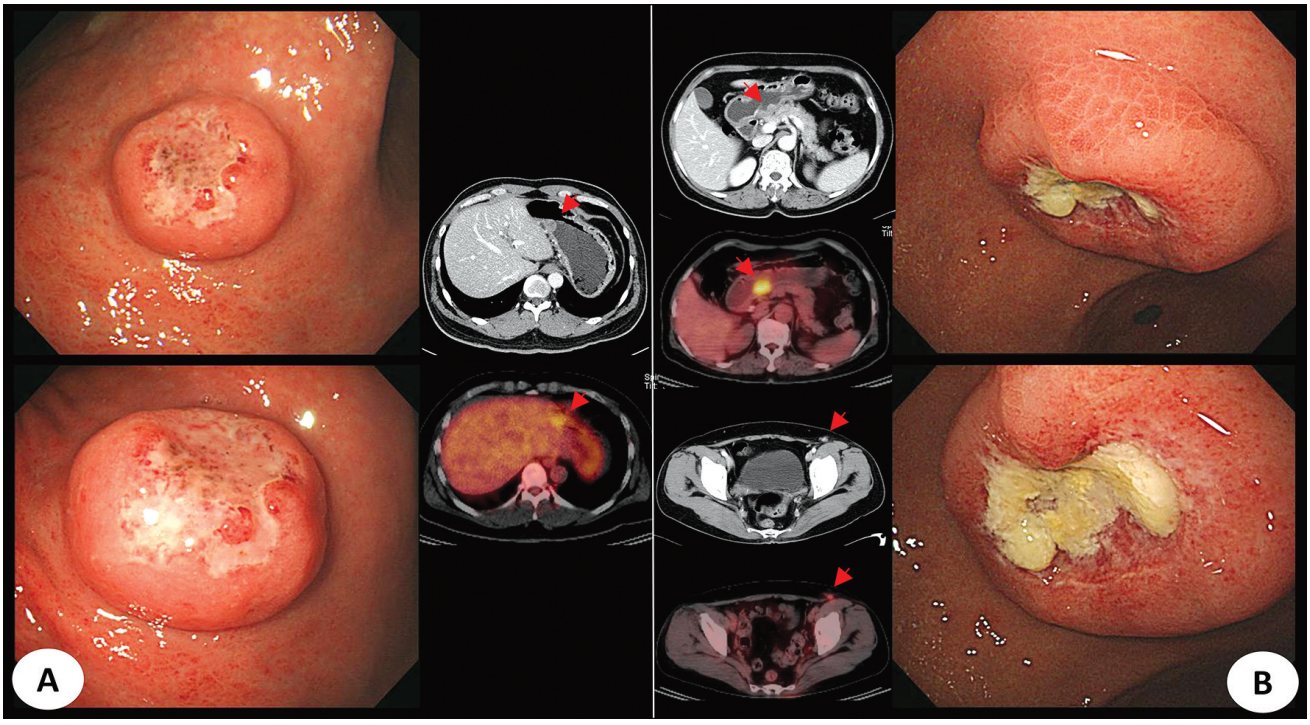

Fig. 3. PET-CT features of endoscopically polypoid mass-like lesions in primary DLBCL (A) and secondary DLBCL (B) cases (Abbreviation. PET-CT, positron emission tomography-computed tomography; DLBCL, diffuse large B-cell lymphoma).

orbit, liver, both adrenal glands, and prostate as well as right subclavian lymph node (Fig. 4). In the last example, two cases were classified under a mixed type endoscopically. All cases were confirmed as diffuse large B-cell lymphoma. In the latter case, lymph node involvement of the whole body including cervical, left mediastinal, both axillary area, aortocaval and both inguinal areas was confirmed in PET-CT (Fig. 5).

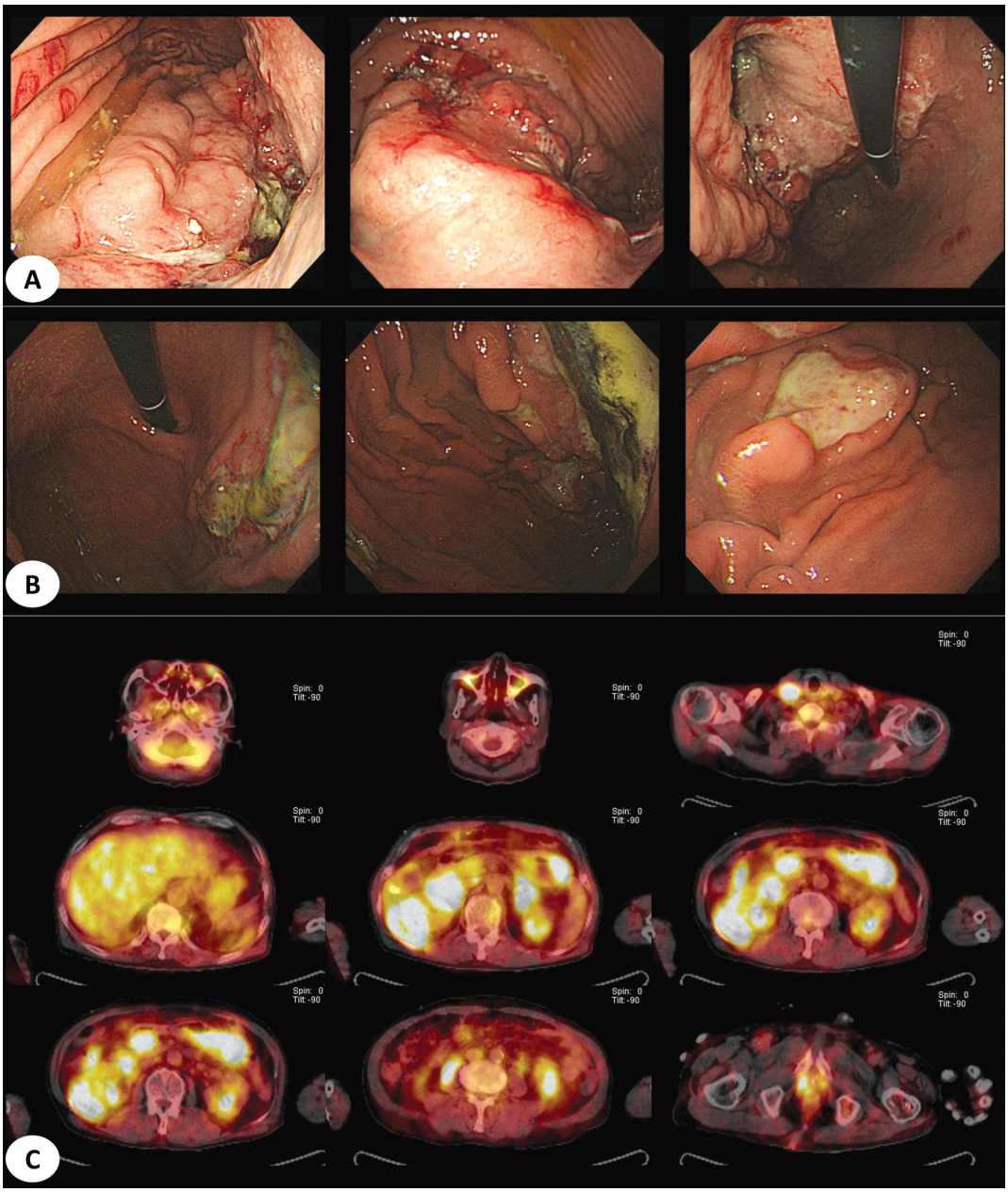

Fig. 4. Endoscopically ulceroinfiltrative lesions of two cases. (A) Primary gastric lymphoma which was diagnosed as mantle-cell lymphoma. (B) Secondary gastric lymphoma which was diagnosed as DLBLC. (C) Multiple involved lymph nodes and organs were observed on PET-CT in same patients of B (Abbreviation. DLBCL, diffuse large B-cell lymphoma; PET-CT, positron emission tomography-computed tomography).

DISCUSSION

In the present study, we identified the clinical differences between primary and secondary lymphomas and the differences in endoscopic morphology associated with malignant gastric lymphoma, except mucosa-associated lymphoid tissue (MALT) lymphoma. Although the clinical features and endoscopic findings of gastric lymphoma were reported, no study reported the com-

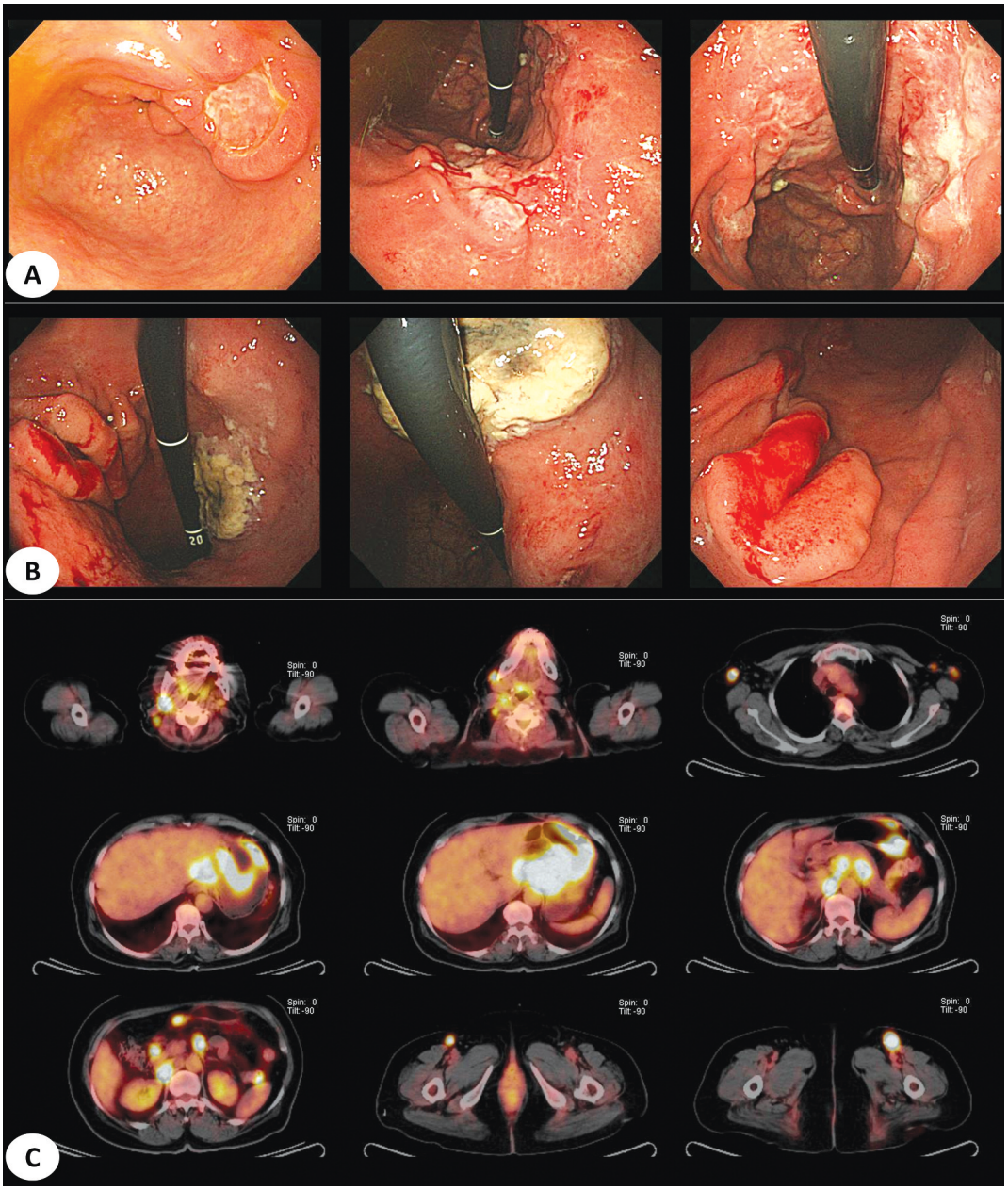

Fig. 5. Two cases of mixed type with two or more endoscopic findings. (A) Primary DLBLC. (B) Secondary DLBLC. (C) Hypermetabolism of stomach and multiple distant lymph node was seen on PET-CT in cases of B (Abbreviation. DLBCL, diffuse large B-cell lymphoma; PET-CT, positron emission tomography-computed tomography).

parison of primary and secondary gastric lymphomas. We found no differences in age and gender between primary and secondary malignant gastric lymphomas. However, cases with fundus involvement were significantly higher in secondary gastric lymphoma. Similar to our study, Kolve et al showed that primary and secondary gastric NHL differed endoscopically, and the fundus was a low-risk factor for primary gastric NHL whereas unifocal growth pattern increased the risk of primary gastric lymphoma.[10]

DLBCL was the most common pathology underlying primary and secondary lymphoma, without any difference in invasion depth, bone marrow involvement, or *Helicobacter pylori* positivity between the two groups. On the other hand, regional lymph node involvement was significantly higher in secondary lymphoma. Based on endoscopic findings, ulceroinfiltrative type was the most common in both groups, and based on first impression of endoscopists, gastric lymphomas were found only in about 40%. According to a report by Cui et al.[11] in a total of 168 patients suspected with endoscopic lymphoma, 35 were diagnosed with gastric lymphoma, with a diagnostic sensitivity of 20.8%, and included 54.3% ulcerative cases. Biopsy revealed DLBCL in 65.8% of the cases. On the other hand, 52.6% of the negative group showed infiltrative type, and histopathological examination showed mostly chronic inflammation (63%).

The first report of gastric lymphoma was classified as mass-forming, diffuse infiltration, with a superficial spread, and considered as unclassified type by Palmer ED[8] in 1950. However, the classification was limited by lack of high-resolution endoscopy at the time. A total of 66 patients were analyzed based on endoscopic findings suggested by Palmer's classification. Of these, 44 patients showed infiltrative findings and 24 patients had exophytic type. The most common endoscopic findings were infiltrative findings.[9] Endoscopic classification was performed to distinguish polypoid, ulcerative, ulcerative-infiltrative, diffuse-infiltrating, and normal findings in a subsequent study by Kolve M et al.[10] Another study by Andriani A et al.[4], identified endoscopic findings involving ulcerative, exophytic, hypertrophic, mucosal petechial hemorrhage, and normal mucosa types. However, this study excluded MALT lymphoma, a type of low-grade lymphoma, and thus did not appear to be a normal mucosa in the endoscopic classification. This normal finding was not included in this study.

Although endoscopic studies of previous gastric lymphoma are few, they reveal the following features. Ghimire P et al.[1] concluded that endoscopic evaluation failed to distinguish AGC and gastric lymphoma, although ulceration, diffuse infiltration, and polypoid mass were non-specific but representative findings. Ge et al.[12] analyzed 87 patients with gastrointestinal lymphoma including 46 gastric cases and found that antrum (43.5%) was the most involved. However, more than half of the patients included in this study were MALT patients, suggesting a difference in prognosis between malignant gastric lymphoma and early MALT lymphoma. In study of Zeggai

et al.[13] lymphoma was divided into high and low grades in the Western world. However, ulcerative lesions were the most common macroscopic features regardless of grade, gastric or intestinal lesions. In a case report, Li and Jiang[14] reported hyperemic changes or glass-like submucosal lesions in mantle-cell lymphoma. In our study, ulceroinfiltrative type was the most common, and erosion and polypoid types were similar to those reported in previous studies.

This study has several limitations. First, this study included only 48 patients with malignant lymphoma, excluding MALT lymphoma, in gastric biopsy. Therefore, as the number of patients increases, additional differences in primary and secondary cases can be expected. Second, this study is a retrospective analysis. Therefore, investigations such as *Helicobacter pylori* test were not performed. However, because it is a rare disease, it may be difficult to conduct prospective analysis. Finally, based on the gastric lymphoma progression, cases involving the initial diagnosis of primary gastric lymphoma may be classified as secondary gastric lymphoma in the future. However, because of the limited number of studies investigating endoscopic findings of gastric lymphoma, our analysis represents a meaningful approach.

In conclusion, malignant gastric lymphoma is not easy to distinguish from advanced gastric cancer. Moreover, endoscopic differentiation of primary and secondary gastric lymphoma is difficult. However, when the lesion is present in the fundus, we keep in mind the possibility of secondary gastric lymphoma.

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Efficacy of Evolocumab in Patients with Hypercholesterolemia

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Objectives: The FOURIER trial reported that inhibition of PCSK9 with evolocumab on a background of statin therapy lowered low-density lipoprotein (LDL) cholesterol levels to a median of 30 mg per deciliter (0.78 mmol per liter) and reduced the risk of cardiovascular events. Here, we report data from a single center focusing on the effect of a PCSK9 inhibitor antibody on hyperlipidemia.

Methods: We enrolled 29 hypercholesterolemia patients who had LDL cholesterol levels ≥ 70 mg per deciliter or non-HDL cholesterol ≥ 100 mg per deciliter and were divided into two groups (placebo n = 14, evolocumab n = 15), and participated in a 72 - 96 week, randomized, double-blind, placebo-controlled trial with statin therapy. Patients were randomly assigned to receive evolocumab (140 mg every 2 weeks or 420 mg monthly) or matched placebo via subcutaneous injection. Lipid changes during follow-up were analyzed.

Results: The median LDL cholesterol level at baseline was 88 mg per deciliter, and the average LDL cholesterol level was 101.8 ± 20.0 mg per deciliter. At 4 weeks, the median LDL cholesterol level was 39 mg per deciliter, and the average LDL cholesterol level was 34.8 ± 51.8 mg per deciliter. Compared to placebo group, the LDL cholesterol levels were significantly reduced after treatment ($P < 0.001$), as well as total cholesterol, ApoB, and ApoB / ApoA1 levels. During follow-up, no discomfort was reported at local injection sites, and no cases of abnormal liver function were observed.

Conclusions: Evolocumab significantly reduced LDL cholesterol levels and was well tolerated.

Key Words: Cholesterol LDL, Hypercholesterolemia, Proprotein convertase, Subtilisin-kexin type 9

Atherosclerosis is a major pathophysiological mechanism that can promote the development of cardiovascular and cerebrovascular diseases. Although statin therapy has been a mainstay of treatment for many years, some patients have experienced issues such as statin intolerance. As an important member of the proprotein convertase subtilisin/kexin type 9 (PCSK9) family, PCSK9 can target and bind the low-density lipoprotein receptor (LDLR) to influence lipid metabolism and has become an attractive target for lipid-regulating therapies and atherosclerosis intervention in recent years.

PCSK9 is a member of the family of preprotein-invertase and was first identified in rabbit aortic tissue by Seidah et al[1] in 2003. PCSK9 is the third gene that has been found to be associated with autosomal dominant familial hypercholesterolemia.[2] Studies have shown that PCSK9 can bind to LDLR in liver cells and

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guide its internalization to lysosomal degradation, thereby weakening the ability of the liver to metabolize LDL cholesterol and up-regulating LDL cholesterol levels.[3] However, PCSK9 inhibitor antibodies can specifically target PCSK9 and block its binding with LDLR, thereby increasing the clearance rate of LDL cholesterol and reducing LDL cholesterol levels.[4] PCSK9 not can only degrade LDLR and increase LDL cholesterol levels, it also has other biological functions such as contributing to the development of the nervous system and inducing apoptosis in nerve cells.[5]

Abifadel et al.[6] identified certain mutations in the PCSK9 gene that were associated with elevated serum LDL cholesterol levels and premature coronary heart disease (CHD), as well as certain mutations that were associated with low serum LDL cholesterol levels.[7] Over time, further research has shown that mutations associated with elevated serum LDL cholesterol levels are gain-of-function (GOF) mutations while those associated with low serum LDL cholesterol levels are loss of function (LOF) mutations. Strikingly, subjects with heterozygous LOF mutations exhibit lower serum PCSK9 levels and as much as an 88% reduction in the incidence of CHD over a 15-year period compared with noncarriers.[8] Moreover, despite a complete loss of PCSK9 and associated very low serum LDL cholesterol levels, two subjects who had been identified with compound heterozygote LOF mutations appeared healthy.[9]

LDL cholesterol is a well-established and modifiable risk factor for cardiovascular disease and monoclonal antibodies that inhibit PCSK9 have emerged as a new class of drugs that effectively lower LDL cholesterol levels.[4] Evolocumab is a human monoclonal antibody that has been reported to reduce LDL cholesterol levels by approximately 60%.[10-14]

The purpose of this study was to investigate the efficacy and safety of evolocumab in Korean patients with hypercholesterolemia.

MATERIALS AND METHODS

A 72-96 weeks, randomized, double-blind, placebo-controlled (n = 14) study of evolocumab (n = 15) was conducted between July 2014 and May 2016 with 29 hypercholesterolemia patients who had LDL cholesterol ≥ 70 mg per deciliter or non-HDL cholesterol levels ≥ 100 mg per deciliter with statin therapy (moderate intensity was defined with atorvastatin 10-20 mg or rosuvastatin 5-10 mg; high intensity was defined with atorvastatin 40-80 mg or rosuvastatin 10-20 mg) in our single center, and no patients interrupted statin therapy. Patients were randomized equally and evolocumab or placebo was administered subcutaneously every 4 weeks and lipid changes were assessed. Eligible patients were randomly assigned in a 1:1 ratio to receive subcutaneous injections of evolocumab (either 140 mg every 2 weeks or 420 mg every month, according to patient preference) or matching placebo.

Study visits were scheduled at screening and day 1, and then at weeks 2, 4, 8, and 12. Patients

received subcutaneous evolocumab or placebo on day 1 and at weeks 4 and 8 (3 doses). Blood samples for all assessments were collected after an overnight fast (water only) and analyzed by a central laboratory. After screening, investigators, site staff, and study team members were blinded to all assessment results.

The institutional review board or independent ethics committee at each site approved the protocol and informed consent form. All patients provided written informed consent before study procedures were performed.

Statistical methods: Continuous variables are presented as mean ± standard deviation (SD) and categorical variables are presented as N (%). To determine whether differences between CAD cases and controls were significant for continuous and categorical variables, Student t-test and chi-squared test were used, respectively. All statistical analyses were two-sided and performed with SPSS (Version 22.0, SPSS Inc., Chicago IL, USA), with the threshold for significance set at $P < 0.05$ for all analyses performed.

RESULTS

Of the 36 patients screened, 29 were randomized (evolocumab n = 15; placebo n = 14) (Table 1). Seven patients did not meet the criteria and were excluded from the analyses. Table 1 shows that the patients' characteristics at baseline were similar among the two groups, and most of the enrolled patients were men, but evolocumab group was older than placebo group, the reason may be caused by single center phenomenon. There was no significant difference in lipid levels between the placebo group and the evolocumab group before administration (Table 2). The median LDL cholesterol level at baseline was 88 mg per deciliter, and the average of LDL cholesterol level was 101.8 ± 20.0 mg per deciliter. At 4 weeks, the median LDL cholesterol level was 39 mg per deciliter, and the average of LDL cholesterol level was 34.8 ± 51.8 mg per deciliter, compared to placebo group the LDL cholesterol levels significantly decreased after treatment ($P < 0.001$) (Table 2) (Fig. 1). The median total cholesterol level at baseline was 187 mg per deciliter, and the average total cholesterol level was 179.5 ± 27.8 mg per deciliter. At 4 weeks, the median total cholesterol level was 80 mg per deciliter, and the average total cholesterol level was 105.9 ± 57.7 mg per deciliter, compared to placebo group total cholesterol levels significantly decreased after treatment ($P < 0.001$) (Table 2) (Fig. 2). The median ApoB level at baseline was 107 mg per deciliter, and the average ApoB level was 87.5 ± 27.7 mg per deciliter. At 4 weeks, the median ApoB level was 26 mg per deciliter, and the average ApoB level was 41.5 ± 34.3 mg per deciliter, compared to placebo group ApoB significantly decreased after treatment ($P < 0.001$) (Table 2) (Fig. 2). The median ApoB / ApoA1 level at baseline was 0.59 mg per deciliter, and the average for ApoB / ApoA1 was 0.6 ± 0.2 mg per deciliter. At 4 weeks, the median ApoB / ApoA1 level was 0.16 mg per deciliter, and the average for ApoB / ApoA1 was 0.3 ± 0.2 mg per deciliter, compared to placebo group the ApoB /

Table 1. Study participants' baseline data

Variables	Placebo (n = 14)	Evolocumab (n = 15)	*P*-value
Age, yrs	64.8 ± 7.3	72.9 ± 6.5	0.004
Male	11 (78.6)	14 (93.3)	0.272
BMI, kg/m^2	25.1 ± 4.5	25.6 ± 3.1	0.752
LVEF, %	56.8 ± 13.4	54.4 ± 9.5	0.607
Risk factors, n (%)			
Current smoking	1 (7.1)	1 (6.7)	0.741
HTN	8 (57.1)	6 (40.0)	0.356
Diabetes	6 (42.9)	4 (26.7)	0.300
Medication at enrollment, n (%)			
Aspirin	13 (92.9)	15 (100)	0.292
Clopidogrel	11 (78.60)	11 (73.3)	0.742
ACEI/ARB	3 (21.4)	3 (20)	0.657
CCB	9 (64.3)	13 (86.7)	0.159
Antidiabetic	5 (35.7)	4 (26.7)	0.674
Statin dose	Statin dose		0.996
Moderate intensity	12 (85.7)	14 (93.3)	
High intensity	2 (14.3)	1 (6.7)	
Lipid measurement			
Total cholesterol, mg/dl	164.9 ± 38.4	179.5 ± 27.8	0.246
Triglycerides, mg/dl	121.4 ± 58.0	145.7 ± 58.1	0.270
HDL-C, mg/dl	44.1 ± 11.0	48.7 ± 12.2	0.297
LDL-C, mg/dl	93.4 ± 37.9	101.8 ± 20.0	0.455
Lipoprotein(a), mmol/l	64.6 ± 90.1	62.5 ± 64.4	0.941
ApoA1, g/l	130.9 ± 19.8	139.2 ± 34.7	0.442
ApoB, mg/dl	81.8 ± 17.9	87.5 ± 27.7	0.521
ApoB / ApoA1, mg/dl	0.6 ± 0.1	0.6 ± 0.2	0.810

Data are presented as mean ± SD or number (%).
BMI = body mass index; LVEF = left ventricular ejection fraction; HTN = hypertension; ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin II receptor blocker; CCB = calcium channel blockers; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; ApoA1 = apolipoprotein A1; ApoB = apolipoprotein B.

ApoA1 level significantly decreased after treatment ($P < 0.001$) (Table 2) (Fig. 2). During follow-up, AST and ALT levels remained within the normal range, and no cases of abnormal liver function were found (Fig. 3).

DISCUSSION

The FOURIER study[15] revealed that evolocumab lowered LDL cholesterol levels by 59% from baseline levels as compared with placebo, from a median of 92 mg per deciliter (2.4 mmol per liter) to 30 mg per deciliter (0.78 mmol per liter). Our results showed that the median LDL cholesterol level in the placebo group was 88 mg per deciliter, and the average LDL cholesterol level

Table 2. Laboratory data at baseline and after 4 weeks

Variables	Baseline			4 weeks		
	Placebo (n = 14) (n = 14)	Evolocumab (n = 15)	*P*-value	Placebo (n = 14)	Evolocumab (n = 15)	*P*-value
AST,	23.5 ± 6.5	22.9 ± 5.4	0.777	21.4 ± 5.0	21.2 ± 5.9	0.939
ALT,	24.3 ± 8.9	25.0 ± 14.9	0.878	21.1 ± 11.9	24.0 ± 11.0	0.508
HbA1c	6.3 ± 1.1	6.6 ± 1.0	0.394	6.3 ± 1.1	7.0 ± 1.4	0.162
Total-C	164.9 ± 38.4	179.5 ± 27.8	0.246	165.1 ± 35.9	105.9 ± 57.7	<0.001
Triglyceride	121.4 ± 58.0	145.7 ± 58.1	0.270	123.1 ± 41.3	112.7 ± 48.6	0.541
HDL-C	44.1 ± 11.0	48.7 ± 12.2	0.297	48.1 ± 10.7	48.1 ± 8.6	0.999
LDL-C	93.4 ± 37.9	101.8 ± 20.0	0.455	96.0 ± 30.5	34.8 ± 51.8	<0.001
ApoA1	130.9 ± 19.8	139.2±34.7	0.442	135.9 ± 20.5	137.3 ± 16.5	0.840
ApoB	81.8 ± 17.9	87.5 ± 27.7	0.521	80.1 ± 19.5	41.5 ± 34.3	<0.001
ApoB/ApoA1	0.6 ± 0.1	0.6 ± 0.2	0.810	0.6 ± 0.2	0.3 ± 0.2	<0.001
Lipoprotein(a)	64.6 ± 90.1	62.5 ± 64.4	0.941	60.3 ± 68.0	42.8 ± 46.6	0.470

Data are presented as mean ± SD.
AST = aspartate aminotransferase; ALT = alanine aminotransferase; HbA1c = hemoglobin A1c, Total-C = total cholesterol, other abbreviations as for Table 1.

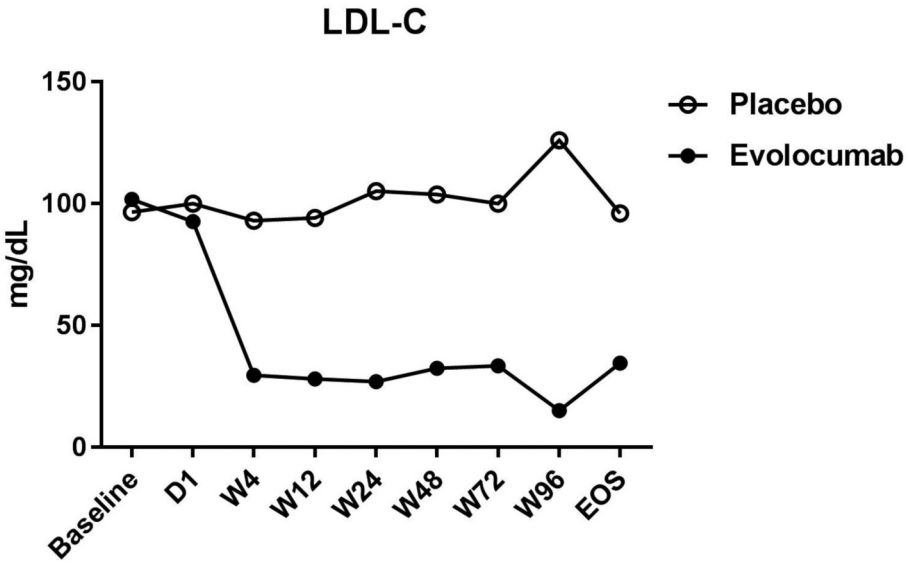

Fig. 1. Effect of Evolocumab on Levels of Low-Density Lipoprotein Cholesterol
EOS = end of study, other abbreviations as for Table 1.

of the placebo group was 101.8 ± 20.0 mg per deciliter. After treatment, the median LDL cholesterol level in the evolocumab group was 39 mg per deciliter, and the average LDL cholesterol level in the evolocumab group was 34.8 ± 51.8 mg per deciliter, with the evolocumab group

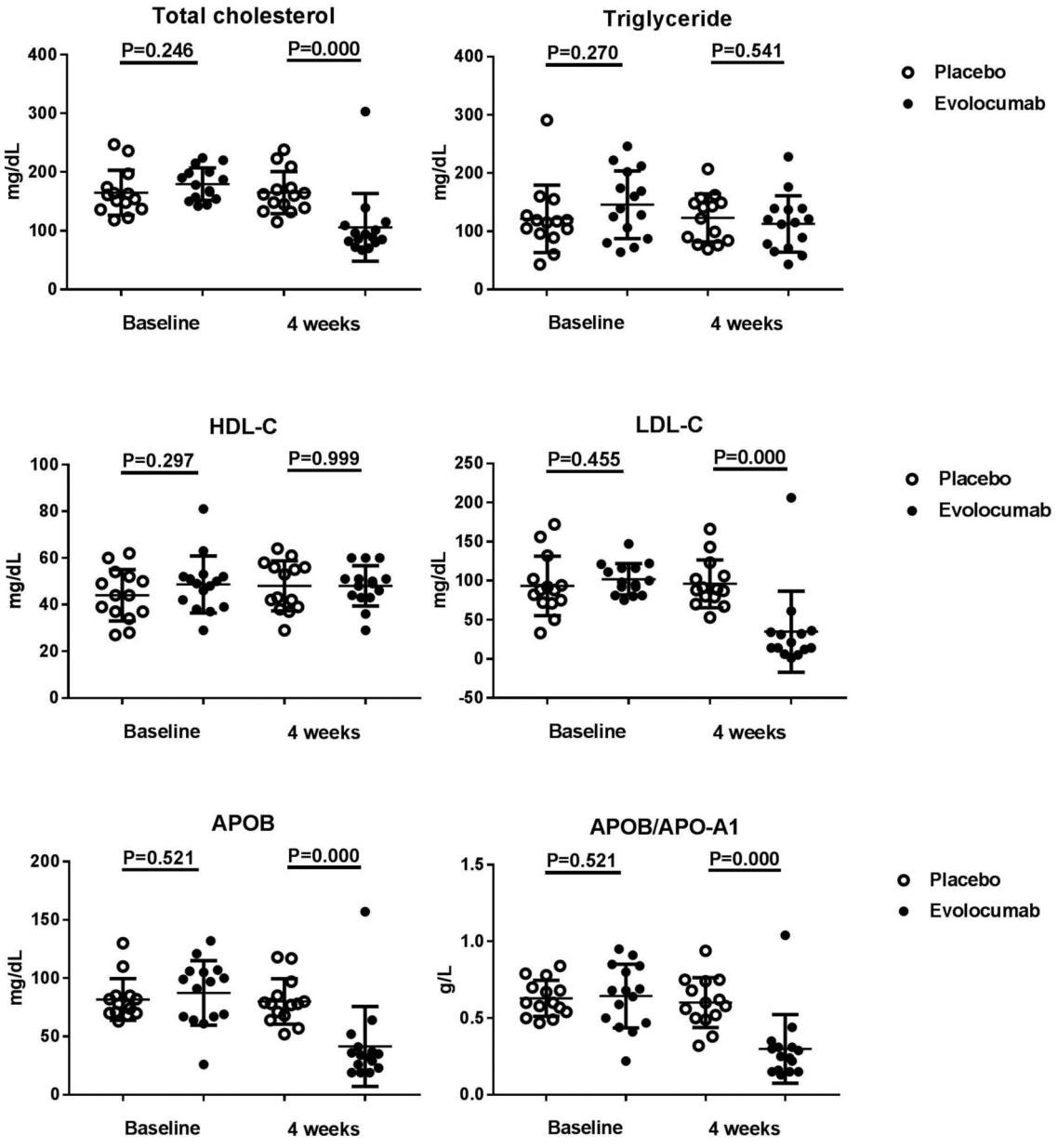

Fig. 2. Changes in Lipid Profile Following Evolocumab Administration
Abbreviations as for Table 1.

showing reduced LDL cholesterol levels by 56% compared to the placebo group. The FOURIER study[15] reported no significant differences in the overall rates of adverse events between the two groups, and injection site reactions were rare, although they were more frequent with evolocumab (2.1% vs. 1.6%). However, there were no adverse reactions during treatment and no injection site reactions observed in our study. The baseline statin dose for the patients in our study was lower than that used in the FOURIER study, as Asian patients are less likely to be prescribed with high-dose statins than their Caucasian counterparts.

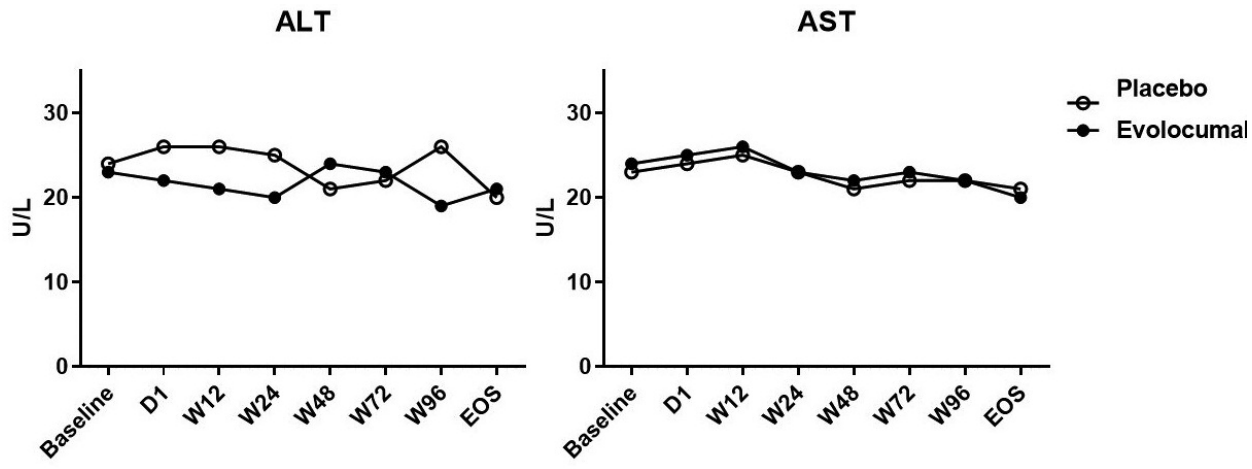

Fig. 3. Changes in Hepatic Enzyme During Follow-up
Abbreviations as for Figure 1 and Table 2.

Statins are the most efficacious agents for alleviating LDL cholesterol levels, although some patients cannot tolerate treatment primarily due to muscle-related side effects, and sometimes higher doses are required to achieve target LDL cholesterol levels.[16] Nevertheless, statin-intolerant patients require more effective LDL cholesterol-lowering therapies. Our findings suggest that evolocumab combined with statin therapy can effectively reduce lipid LDL-C levels.

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Usefulness of Psoas Muscle Cross-Sectional Area in Evaluating Physical Performance in Patients with Liver Cirrhosis

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Objectives: To investigate the relationship between the psoas muscle cross-sectional area and physical performance in patients with liver cirrhosis.

Methods: This study analyzed ambulatory patients with liver cirrhosis aged < 65 years, who underwent abdominal computed tomography (CT) and Short Physical Performance Battery (SPPB) tests from December 2018 to December 2019. A total of 46 patients (36 men, 10 women) were included. In abdominal CT scans, the psoas muscle cross-sectional area (mm^2) was measured at the distal end-plate level of the L4 vertebral body and normalized by dividing by height (m). Physical performance was evaluated using SPPB. A correlation analysis between the psoas muscle cross-sectional area and SPPB was performed. Kruskal-Wallis test was used to determine differences in the psoas muscle cross-sectional area and SPPB according to the Child-Pugh classification. Multiple regression analysis was performed to determine factors affecting SPPB.

Results: The correlation coefficient between the psoas muscle cross-sectional area and SPPB was 0.459 at the $P < 0.01$ level. No difference was observed in the psoas muscle cross-sectional area and SPPB according to the Child-Pugh classification. The psoas muscle cross-sectional area was a factor affecting SPPB in multiple regression analysis.

Conclusions: Abdominal CT is an essential diagnostic tool in patients with liver cirrhosis. Ambulatory patients with liver cirrhosis aged < 65 years could have reduced physical performance. In this study, the psoas muscle cross-sectional area was correlated with physical performance and was a factor affecting physical performance. The psoas muscle cross-sectional area and physical performance should be evaluated in patients with liver cirrhosis.

Key Words: Liver cirrhosis, Physical performance, Psoas muscles

Liver cirrhosis is a representative disease causing inadequate nutrition and protein depletion, with a chronic disease course.[1] In patients with liver cirrhosis, micronutrient deficiency and increased leptin and pro-inflammatory cytokines lead to a decrease in taste acuity and appetite. Moreover, reduced intestinal absorption results in hypermetabolic conditions, which are associated with increased energy consumption and high protein catabolism. As a result, skeletal muscle loss and reduced physical performance occur.[2] Therefore, it is important to evaluate skeletal muscle and physical performance in patients with liver cirrhosis. Several studies have

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evaluated skeletal muscle mass using computed tomography (CT). Kalafateli et al. and Kim and Jang evaluated skeletal muscle mass using cross-sectional muscle areas measured in CT scans.[2,3]

Among the various methods for evaluating skeletal muscle mass using CT scans, measurement of the psoas muscle has recently attracted attention. One previous study showed that the psoas muscle area significantly increased after the improvement of liver cirrhosis in patients who underwent a stent procedure.[4] Another study reported a negative correlation between the psoas muscle area and posttransplant mortality.[5] The psoas muscle has also been reported to be useful in estimating skeletal muscle mass in patients with liver cirrhosis, as well as for nutritional and metabolic status assessment.[6] In another study, the psoas muscle thickness of patients with liver cirrhosis was highly correlated with the skeletal muscle index (SMI) and was reported to predict mortality in this patient population.[7] Similarly, various other studies have demonstrated the clinical importance of the psoas muscle in patients with liver cirrhosis.

Physical performance is one of the important factors predicting the prognosis of liver diseases. In previous studies, higher physical performance was observed in nonhospitalized patients with liver cirrhosis than in hospitalized patients.[8] Moreover, the Short Physical Performance Battery (SPPB) was found to be useful for the development of a frailty index for predicting mortality in patients with end-stage liver disease.[9]

The cross-sectional area of the psoas muscle can be easily obtained through abdominal CT, which is an essential imaging method in patients with liver cirrhosis. Assessing muscle mass using the psoas muscle cross-sectional area and analyzing its association with clinical parameters that can affect physical performance are useful. However, few studies have investigated the relationship between the psoas muscle cross-sectional area and physical performance in patients with liver cirrhosis. Moreover, few studies have been published on the difference of the psoas muscle cross-sectional area and physical performance according to the severity of liver cirrhosis.

The purpose of this study was to investigate the relationship between the psoas muscle cross-sectional area, which is a quantitative parameter of muscle mass determined using abdominal CT scans, and physical performance. Moreover, we also investigated the relationship between physical performance and other clinical parameters that can affect physical performance in liver cirrhosis, including SMI, grip strength, and serum albumin level.

MATERIALS AND METHODS

Patients

This study involved a retrospective review of medical charts of patients diagnosed with liver cirrhosis at OO university hospital between December 2018 and December 2019. The inclusion criteria were as follows: (1) an initial diagnosis of liver cirrhosis based on abdominal CT and

liver biopsy results; (2) ability to ambulate; and (3) age < 65 years, to rule out low physical performance due to aging. The exclusion criteria were as follows: (1) advanced liver cancer at the time of liver cirrhosis diagnosis; (2) a history of neurologic disease that may affect physical performance; (3) active encephalopathy due to liver cirrhosis; and (4) inability to maintain a neutral anteroposterior position because of a spinal disease, such as scoliosis. The severity of liver cirrhosis was determined according to the Child-Pugh classification.[10] The sample size was obtained using the correlation coefficient between the psoas muscle cross-sectional area and SPPB calculated with MedCalc (MedCalc Software, Ostend, Belgium), with a power of 0.80 and a significance of 0.01. This study was approved by the Institutional Review Board of OO university hospital.

Psoas muscle cross-sectional area

In this study, the abdominal CT scans of all patients, obtained using the same criteria and methods, were used to measure the psoas muscle cross-sectional area. In a study on the psoas muscle cross-sectional area, the psoas muscle at the L4/5 level had the largest cross-sectional area and showed the highest symmetry.[11] In the current study, we measured the psoas muscle cross-sectional area at the distal end-plate level of the L4 vertebral body, which is close to the L4/5 intervertebral disc. The psoas muscle cross-sectional area on the right side was measured by two trained operators using FIJI/ImageJ software (Laboratory for Optical and Computational Instrumentation, University of Wisconsin-Madison, Madison, WI, USA) (Fig. 1). Finally, the average of the psoas muscle cross-sectional area measurements by the two operators was calcu-

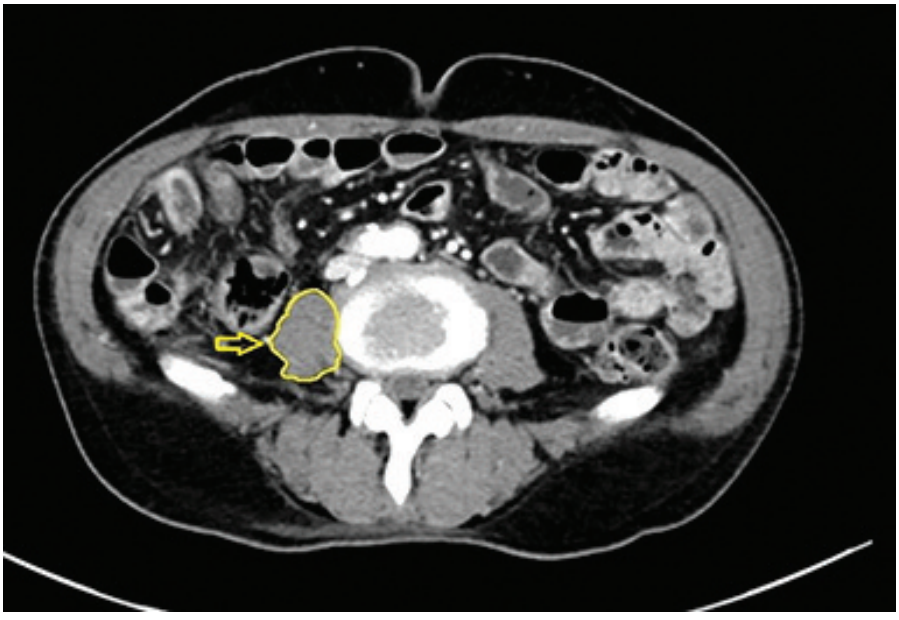

Fig. 1. Measurement of the psoas muscle cross-sectional area (arrow) at the distal end-plate level of the L4 vertebral body.

lated and normalized by dividing by the patient's height (m).

Skeletal muscle index

To obtain the SMI (kg/m^2), the predicted skeletal muscle mass (kg) was divided by height squared (m^2). As this was a prospective study, bioimpedance analysis (BIA) was selected to measure the predicted skeletal muscle mass because it is quick to perform, cost-effective, and safe compared with other evaluation methods. BIA was performed using an Inbody S10 machine (Inbody, Seoul, South Korea). When performing BIA, electrodes were attached to the first and third fingers, as well as to the ankles of the patient in the supine position. The SMI obtained using BIA is considered to indicate low muscle mass when the value is ≤ 8.87 kg/m^2 in men and ≤ 6.42 kg/m^2 in women, according to a representative study on SMI measurement using BIA.[12]

Muscle strength

Muscle strength (kg) was evaluated by measuring the isometric handgrip strength of the dominant hand using a Jamar hydraulic hand dynamometer (Jamar, Chicago, IL, USA). Tests were performed two times at 5-min intervals, and the higher value of the two tests was adopted for the analysis.

Physical performance

SPPB was used as a measure of physical performance. SPPB consists of balance, gait, strength, and endurance, assessed by examining a patient's ability to stand with the feet together in side-by-side, semi-tandem, and tandem positions; the time to walk 4 m; and the time to rise from a chair and return to the seated position five times.[12] SPPB uses the total score of balance, gait speed, strength, and endurance with a minimum of 0 points and maximum of 4 points in each component.

Nutritional status assessment

The biochemical parameters used for nutritional status assessment in patients with liver diseases are albumin, pre-albumin, retinol-binding protein, and serum total protein.[13] In this study, we measured serum albumin, which is used as a criterion of liver cirrhosis severity and is commonly assessed in clinical practice. Serum albumin was considered abnormal when the level was < 3.5 g/dL, and the patients were divided into the normal and abnormal groups accordingly.

Statistical analysis

Reliability analysis was performed to check the inter-rater reliability of the psoas muscle cross-sectional area. Bivariate correlation analysis was performed to investigate the correlation between SPPB and serum albumin, age, body mass index, and SMI. The Mann-Whitney U-test was performed to determine the effect of sex and serum albumin on the SPPB test results. A Kruskal-Wallis test was performed to determine differences in the psoas muscle cross-sectional area, SMI, serum albumin, and SPPB according to the severity of liver cirrhosis. Univariate analysis was per-

formed to select independent variables in multiple regression analysis. Finally, multiple regression analysis was performed to determine the factors affecting SPPB.

RESULTS

A total of 46 patients (36 men, 10 women) were finally included in this study (the sample size calculated by MedCalc was 50 patients). The number of patients with Child-Pugh class A, B, and C was 25, 8, and 13, respectively (Table 1). The inter-rater correlation coefficient was 0.959, with a P-value of 0.000. In bivariate correlation analysis, the psoas muscle cross-sectional area was significantly correlated with SPPB (r = 0.459, $P < 0.01$). SPPB had no significant correlation with SMI, age, or body mass index (Table 2). In the Mann Whitney U-test, SPPB had a significant difference according to serum albumin ($P = 0.003$) but showed no significant difference according to sex (Table 2). The mean value of serum albumin was statistically significant according to the severity of liver cirrhosis; however, the differences in the mean values of the psoas muscle cross-sectional area, SMI, and SPPB according to liver cirrhosis severity were not statistically significant in the Kruskal-Wallis test (Table 3). The psoas muscle cross-sectional area, grip strength, and serum albumin showed significance in univariate analysis. In multiple regression analysis, all independent variables were entered into the equation. The psoas muscle cross-sectional area, grip strength, and serum albumin were identified as factors affecting SPPB (Table 4).

Table 1. Demographic and baseline clinical characteristics

Variable	Value	
	Men (n = 36)	Women (n = 10)
Psoas muscle cross-sectional area (mm^2/m)	652 ± 126	456 ± 113
Skeletal muscle index (kg/m^2)	11.59 ± 1.78	10.02 ± 1.08
Grip strength (kg)	31.5 ± 8.8	17.8 ± 4.2
Short Physical Performance Battery	10.9 ± 1.9	9.2 ± 2.6
Serum albumin (g/dL)	3.38 ± 0.72	3.11 ± 0.86
Body mass index	23.46 ± 3.67	25.74 ± 6.0
Age (years)	56.2 ± 6.2	55.8 ± 6.8
Child-Pugh classification		
Class A	21 (58%)	4 (40%)
Class B	5 (14%)	3 (30%)
Class C	10 (28%)	3 (30%)

Values are presented as mean ± standard deviation or number (%).

Table 2. Comparison of factors with SPPB

	SPPB
Bivariate correlation analysis	Correlation coefficient
Psoas muscle cross-sectional area	0.459**
Serum albumin	0.415**
Grip strength	0.672**
Age	-0.710
Body mass index	-0.810
Skeletal muscle index	0.018
Mann-Whitney U-test	*P*-value
Sex	0.093
Serum albumin	0.003**

SPPB, Short Physical Performance Battery.
** $P < 0.01$.

Table 3. Comparison of factors according to liver cirrhosis severity

	Child-Pugh classification			*P*-value			
	Class A (n = 25)	Class B (n = 8)	Class C (n = 13)	Across three groups	Between two groups		
PMCSA (mm^2/m)	616 ± 141	579 ± 184	613 ± 146	0.674	A vs. B	A vs. C	B vs. C
SMI (kg/m^2)	11.32 ± 1.89	10.62 ± 1.32	11.46 ± 1.80	0.370	A vs. B	A vs. C	B vs. C
Serum albumin (g/dL)	3.58 ± 0.72	2.90 ± 0.70	3.08 ± 0.73	0.042[a]	A vs. B[b]	A vs. C	B vs. C
Grip strength (kg)	29.9 ± 10.0	27.2 ± 12.0	26.6 ± 8.2	0.601	A vs. B	A vs. C	B vs. C
SPPB	10.56 ± 1.85	9.87 ± 2.74	9.84 ± 2.07	0.359	A vs. B	A vs. C	B vs. C

Values are presented as mean ± standard deviation.
[a] $P < 0.05$
[b] $P < 0.05$ by Kruskal-Wallis test for continuous variables and Mann-Whitney U-test for post-hoc analysis.
PMCSA, psoas muscle cross-sectional area; SMI, skeletal muscle index; SPPB, Short Physical Performance Battery.

Table 4. Multiple regression analysis of factors correlated with SPPB

	Standardized ß	*P*-value	*Adjusted* R^2
Psoas muscle cross-sectional area	0.269	0.035*	0.463
Grip strength	0.335	0.016*	
Serum albumin	0.300	0.021*	

*$P < 0.05$ by multiple regression analysis.

DISCUSSION

In this study, the psoas muscle cross-sectional area showed a statistical correlation with SPPB, and was also identified as a factor affecting physical performance in patients with liver cirrhosis in multiple regression analysis. In addition, the psoas muscle cross-sectional area was a statistically more obvious factor than albumin. The SMI had no significant correlation with physical performance. Age, body mass index, and sex also had no significant correlation with physical performance. Therefore, the psoas muscle cross-sectional area is a useful parameter for evaluating physical performance in patients with liver cirrhosis.

Gu et al. previously suggested the clinical usefulness of the psoas muscle thickness for diagnosing sarcopenia in patients with liver cirrhosis, and Kim et al. reported that the psoas muscle thickness divided by the patient's height is a useful factor for predicting long-term mortality in patients with liver cirrhosis with ascites.[5,14] Therefore, various evaluations and predictions using the psoas muscle have been attempted in patients with liver cirrhosis. In the current study, the psoas muscle cross-sectional area was identified as a useful factor that was not associated with the severity of liver cirrhosis and was associated with physical performance. Previous studies have demonstrated that the psoas muscle cross-sectional area differs according to age and sex; however, in this study, no differences in the psoas muscle cross-sectional area were found according to age and sex when the area was normalized by the patient's height.[15] Few studies have considered the association between the psoas muscle cross-sectional area and physical performance in ambulatory patients with liver cirrhosis aged < 65 years. Therefore, this study is valuable as a basic preliminary study on this issue.

SPPB was used as a measure of physical performance. According to the Asian Working Group for Sarcopenia, the cutoff value of SPPB was 8 points, based on which nine patients in this study had low physical performance.[16] All patients were aged < 65 years with no specific history that could affect physical performance. Low physical performance may be considered a characteristic sign in patients with liver cirrhosis, raising the importance of physical performance assessment in patients with liver cirrhosis and suggesting that the psoas muscle cross-sectional area is a clinically useful factor for evaluating physical performance.

Further studies are needed in setting the cutoff value of the psoas muscle cross-sectional area normalized by the individual's height depending on age and sex, which can be a clinically useful indicator of physical performance in ambulatory patients with liver cirrhosis aged < 65 years.

In this study, the SMI was obtained using BIA and had no significant correlation with physical performance. In addition, all patients showed a normal SMI, although some patients had low handgrip strength and low physical performance. Prior studies have shown that muscle mass was not associated with physical performance in

weak older adults and that handgrip strength was clinically more important.[17] This study found that muscle mass was not associated with physical performance in patients with liver cirrhosis aged < 65 years.

In this study, nutrition represented by serum albumin was found to affect physical performance. Montano-Loza suggested using the psoas muscle cross-sectional image for nutritional and metabolic assessment in patients with liver cirrhosis and sarcopenia.[6] In this study, the psoas muscle cross-sectional area had no significant association with serum albumin. Several possible reasons for this result can be suggested. First, all patients had no sarcopenia. Second, as the rate of muscle mass loss is estimated to be 1–2% per year and the metabolism cycle of serum albumin is 25 days, there was a difference in the rate of change for these parameters.[18-20] Nevertheless, we suggest that there is a need to evaluate physical performance in cirrhotic patients with low serum albumin because serum albumin was an important factor affecting physical performance in this study.

Whereas previous studies have focused on predicting the SMI in patients with liver cirrhosis, the present study is a basic study on factors affecting physical performance and provides evidence on the clinical usefulness of the psoas muscle cross-sectional area.

This study had some limitations. First, the SMI is usually obtained using dual-energy x-ray absorptiometry (DEXA) and BIA; however, we used only BIA in this study. Previous studies have questioned the accuracy of BIA and indicated that DEXA has a higher accuracy than BIA.[21-23] Further studies evaluating the SMI using DEXA may provide clearer results about the correlation between the SMI and physical performance. Second, the value of the psoas muscle cross-sectional area could have been be overstated in this study. In bivariate correlation analysis, grip strength had a higher correlation coefficient with SPPB than the psoas muscle cross sectional area. Therefore, besides the psoas muscle cross-sectional area, focus should also be directed to the clinical importance of grip strength in evaluating physical performance in patients with liver cirrhosis.

Abdominal CT is an essential diagnostic tool in patients with liver cirrhosis. Therefore, the psoas muscle cross-sectional area can be easily obtained in these patients without additional examination and cost requirements. In this study, we found a correlation between the psoas muscle cross-sectional area and physical performance in ambulatory patients with liver cirrhosis aged < 65 years. Therefore, upon the first diagnosis of liver cirrhosis, evaluation of the psoas muscle cross-sectional area using abdominal CT is important. Furthermore, it is also important to evaluate physical performance in patients with liver cirrhosis with low psoas muscle cross-sectional area even if the SMI is normal. These evaluations may enable the early detection of deterioration of physical performance and allow planning immediate rehabilitation.

CONFLICT OF INTEREST

The authors report no potential conflicts of interest relevant to this article.

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A Case of Trisomy 9 Mosaicism Confirmed by Microarray Test

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Trisomy 9 mosaicism syndrome is a rare chromosomal abnormality with a high incidence of natural abortion and perinatal death. This syndrome is characterized by intrauterine growth retardation, mental retardation, craniofacial dysmorphism including a prominent nasal bridge with a short root and a fish-shaped mouth with thin lips, skeletal abnormalities, congenital heart defects, and genital abnormalities. The incidence and severity of malformations depend on the percentage of trisomic cells in the different tissues. We report a neonate who had the characteristic features of trisomy 9 syndrome with dysmorphic features including micrognathia, microcephaly, a low-set and malformed ear, a prominent lip, and cardiac defect. No chromosomal abnormalities were detected on a routine peripheral blood chromosomal analysis; however, a chromosomal abnormality with trisomy 9 mosaicism (low-level mosaic type) was detected on genetic tests. This is thought to be due to the low proportion of trisomic cells, and for this reason, the patient in this case shows a better prognosis than four patients previously reported in Korea, they were all diagnosed by peripheral blood chromosome testing.

Key Words: Fluorescence in situ hybridization, Microarray test, Trisomy 9 mosaicism

Trisomy 9 mosaicism is a rare chromosomal abnormality. Since its first mention in 1973 by Haslam et al.,[1] approximately 150 cases have been reported worldwide.[2] In Korea, Kim et al. described a case of trisomy 9 mosaicism, which had been mistaken as Smith–Lemli–Opitz syndrome.[3]

Trisomy 9 mosaicism is characterized by intrauterine growth retardation (IUGR), mental retardation, craniofacial deformities, skeletal abnormalities, congenital heart defects, and genital abnormalities. The incidence and severity of the accompanying malformations and mental retardation correlate with the percentage of trisomic cells.[4,5]

Here, we report a case of a neonate with trisomy 9 mosaicism that, was confirmed by single nucleotide polymorphism array and fluorescence in situ hybridization tests, even though the results of the initial peripheral blood genetic testing were normal.

CASE

The male infant was delivered by emergent ce-

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sarean section at 36 and 5/7 weeks of gestation. The patient was the first child of non-consanguineous parents. The mother of the patient was 32 years old and denied that she had any exposure to radiation or medication during pregnancy. She was diagnosed with hypothyroidism 2 years earlier, but no medication was administered. Furthermore, she had no specific family history associated with any hereditary disorder or chromosomal abnormalities. The mother had undergone regular check-ups at a local gynecology clinic. During the routine prenatal examination at the gynecology clinic, UGR with oligohydramnios was observed beginning in the third trimester; thus, she was referred to our hospital. However, on arrival at our out-patient clinic, we detected fetal heart rate deceleration ion and poor variability. Therefore, emergent caesarian section was indicated. A prenatal cytogenetic study was not performed.

The weight of the neonate was 1,350 g, length was 41.0 cm, and head circumference was 29.5 cm, all of which were less than the 10th percentile. The patient was admitted to our neonatal intensive care unit shortly after birth due to respiratory difficulties. Upon presentation, the patient had a dysmorphic face with a broad nasal root; small fish-shaped mouth with thin lower lips; retro-micrognathia; low-set, malformed and posteriorly rotated ears; microcephaly; and plagiocephaly (Fig. 1). Physical examination revealed a grade II systolic murmur at the left suprasternal notch.

The initial chest radiograph showed decreased

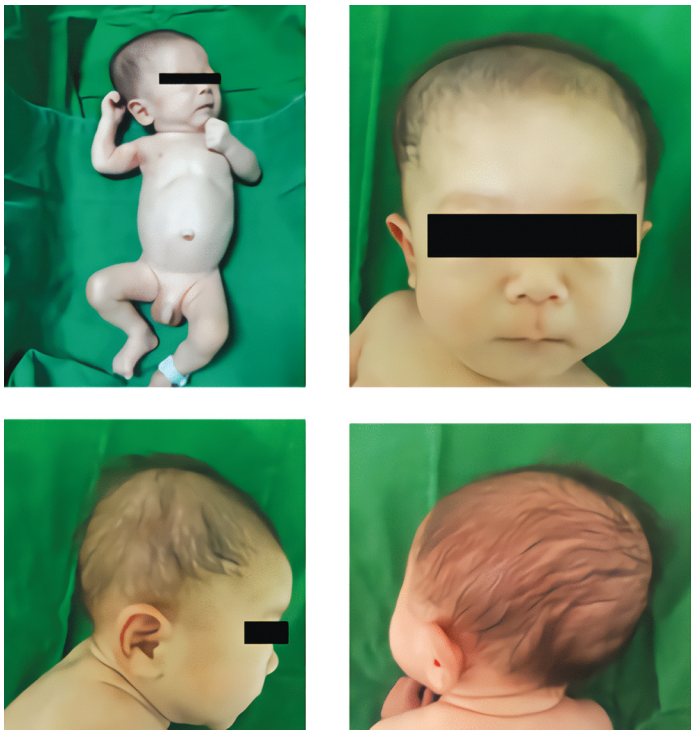

Fig. 1. General appearance of the patient shows an asymmetric head(Plagiocephaly), low-set ears, small mouth, and thin lips.

lung volume with air bronchogram. The patient was initially treated with surfactant following the INSURE technique, and then, a nasal positive pressure ventilator was applied for respiratory support. Results of the routine laboratory test were as follows: white blood cell count, 4,400/mm^3; hemoglobin level, 16.7 g/dL; platelet count, 99,000/mm^3; $Na^+/K^+/Cl^-$ concentrations, 139/4.5/105 mEq/L; total protein/albumin level, 4.9/3.3 mg/dL; BUN/Cr concentration, 11.7/0.85 mg/dL; aspartate aminotransferase/alanine aminotransferase level, 48/5 U/L; and C-reactive protein level, 0.01 mg/dL. Complete blood tests showed no abnormalities other than mild thrombocytopenia. Several tests were performed to assess for the presence of metabolic disorders and congenital infection. Metabolic evaluation showed normal results. Moreover, the patient tested negative for herpes simplex, cytomegalovirus, rubella, and toxoplasma. Initial brain sonography revealed increased periventricular echogenicity. The abdominal ultrasonographic examination showed no abnormal findings.

On the 2nd day after birth, enteral feeding was initiated, through an orogastric tube and was well tolerated. Full enteral nutrition was achieved by on the 16th day after birth.

On the 3rd day after birth, the patient's echocardiogram showed perimembranous ventricular septal defect (VSD) measuring 4.0 mm, atrial septal defect (ASD) measuring 3.5 mm, and a small patent ductus arteriosus. The follow-up echocardiogram performed on the 17th day after birth revealed a closed ductus arteriosus with the VSD measuring 4.0 mm and the ASD measuring 2.5 mm.

On the 6th day after birth, the patient's platelet count decreased to 6,000/mm^3; hence, intravenous immunoglobulin (IVIG) was administered (1 g/kg/d^1 for 2 days) under suspicion of neonatal allo-immune thrombocytopenia because mother's platelet count was normal (284,000/mm^3). Shortly after the initiation of IVIG treatment, the patient's platelet count increased to 38,000/mm^3 and then gradually returned to a normal level 20 days after performing this series of laboratory tests. Since then, his platelet count has remained normal.

On the 13th day after birth, the direct bilirubin level of the patient´increased to 1.64 mg/dL, and his total bilirubin was 8.64 mg/dL. Work-ups for cholestasis including abdominal sonography, viral marker and other blood tests (TORCH and thyroid function test) all showed negative findings; hence, the patient was treated under the assumption of parenteral nutrition – associated cholestasis (PNAC). Therefore, we applied a more aggressive enteral nutritional therapy including a rapid increase in the amount of enteral feeding, a limitation on the maximum intake of parenteral amino acid solution, and removal of copper and manganese from the parenteral nutrition solution in combination with ursodeoxycholic acid (UDCA) treatment. On the 62nd day after birth, direct bilirubin had normalized and UDCA treatment was discontinued.

Brain magnetic resonance imaging performed on the 63rd day after birth showed on abnormal findings.

After gradual weaning off the ventilator, respiratory support was discontinued on the 13th day after birth. On the 15th day after birth, bottle feeding was started; however, it took a while for the patient to tolerate bottle feeding due to feeding hypoxia. Finally, full bottle feeding was achieved on the 60th day after birth. On the 77th day after birth, the patient was discharged from the hospital with the following assessment findings: weight, 2,850 g (less than the 10th percentile); height, 46 cm (less than the 10th percentile); and head circumference, 36 cm (10–25th percentile).

Genetic testing

Chromosome tests were performed on peripheral blood lymphocytes based on the suspicion of chromosomal abnormalities due to the presence of IUGR, facial dysmorphism, and cardiac defects. A balanced translocation was found from the analysis of 20 dividing cells obtained by culturing from peripheral blood (46, XY, t(2;9)(p22;q33)). Microarray analysis showed a trisomy 9 mosaicism. Microarray analysis was performed using the CytoScan 750K high-resolution single nucleotide polymorphism (SNP) array (Affymetrix, Santa Clara, CA). The array contains > 750,436 copy number variant markers, including 200,436 genotype-able SNP probes and 550,000 non-polymorphism probes. All data were visualized and analyzed using the Chromosome Analysis Suite software package (Affymetrix, USA). The analysis showed a translocation 46,XY,t(2;9)(p22;q39). Based on a comparison with human reference genome 37 (NCBI37/hg19) at the National Center for Biotechnology, a trisomy 9 mosaicism and a 567 Kb duplication which was located in 3q26.1 [arr 3q26.1 (164876489_165443782)x3] were identified (Fig. 2). The fluorescence in situ hybridization (FISH) test showed 10-15% of trisomy 9 cells among 200 cells (Fig. 3).

Outcome

The patient is doing relatively well after discharge. On the last outpatient clinic follow up, the patient was 8 months of age. He was feeding with a bottle and had recently started a soft diet. The VSD had reduced to 1mm in size and there were no symptoms of heart failure, therefore the defect is being observed without surgery. Development of the patient was suitable for a normal 6 to 7 old infant, such as making continuous, vowel and, consonant sounds, and responding to his name. Additionally, he could roll supine to prone and sit down with minimal support. His body measurements were as follow; body weight 6.5kg (< 10th percentile), height 67cm (< 10th percentile), and head circumference 42.0cm (10-25th percentile).

DISCUSSION

A total of 150 cases of chromosome 9 trisomy mosaicism have been reported since it was first reported in 1970 by Haslam et al.[1] In Korea, Jeon et al. reported the first case of trisomy 9 syndrome in 1998.[6] Kim et al. described the first case of trisomy 9 mosaicism, which was mistaken as Smith–Lemli–Opitz syndrome in 2001.[3]

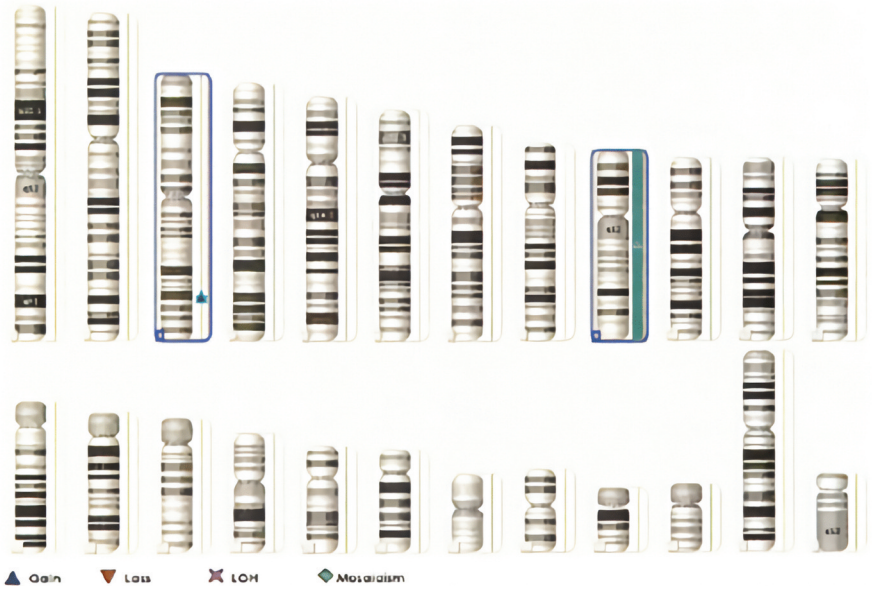

Fig. 2. Microarray shows 3q26.1 region duplication and trisomic chromosome9.

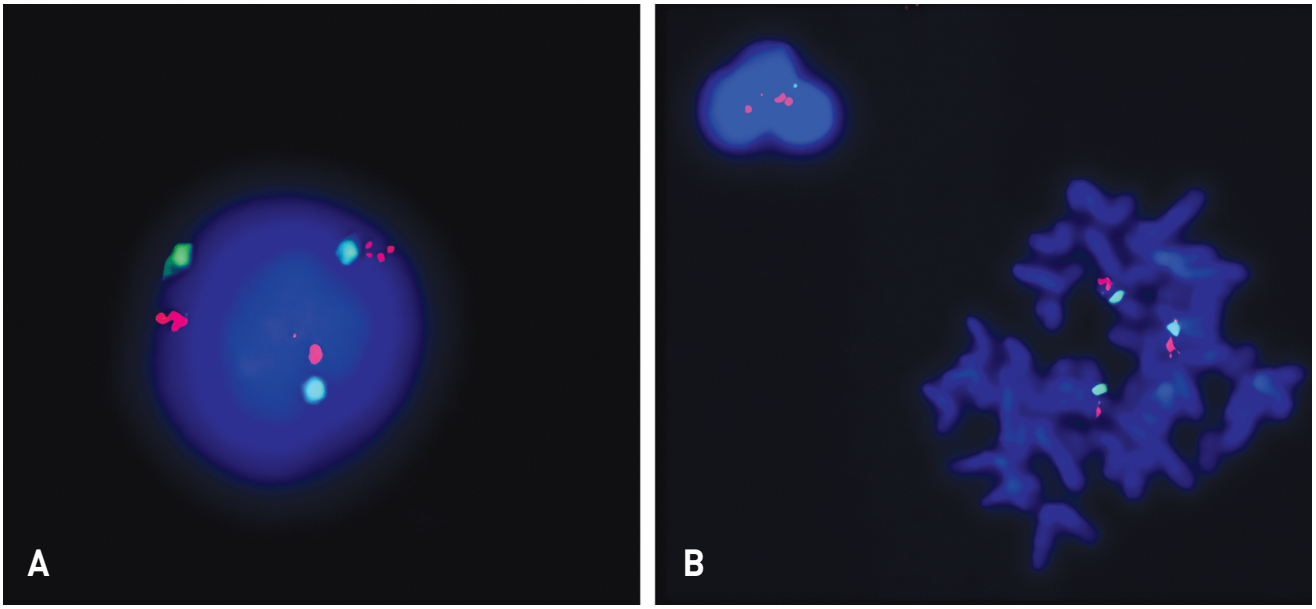

Fig. 3. Results of analysis of lymphocytes in peripheral blood with 9p (red) and 9p (green) FISH probes (Cytocell Ltd, UK) : Three signals are shown in 11% of interphase (A) and metaphase (B) cells, consistent with the diagnosis of mosaic trisomy 9

Since then, only 3 additional cases have been reported: Lee et al.,[7] Kim et al.,[8] and Na et al.[9] in 2002, 2003, and 2016, respectively. Trisomy 9 is subdivided into two types: the complete type, with one additional complete chromosome 9; and the partial type, with a short arm or a part of the long arm of chromosome 9. The partial type is more common than the complete type; in Korea,

cases of partial trisomy 9 have been reported in 1995[10] and 2000[11]

The complete type is further subdivided into mosaic type and non-mosaic type. Most of the fetuses with non-mosaic type have been aborted or died shortly after birth.[4] The average survival time of neonates with non-mosaic type is less than 9 days. The mosaic type is more common than the non-mosaic type and has a better survival rate. It has been reported that some infants with low -level mosaic type can survive for more than 2 years.[12]

The clinical features of infants with trisomy 9 mosaicism are diverse, and, less severe than those of complete types. Infants with partial trisomy 9 may survive until adulthood. The degree of malformation is more severe according to the proportion of trisomy 9 cells.[4,5] Clinical manifestations include microcephaly, micrognathia, narrow temples, dysmorphic face including protruding larynx, thick and wide nose, low-set ears, and fish-shaped mouth. It may be accompanied by genitourinary malformations and central nervous system malformations.[12]

The complete type has a higher rate of natural abortion and postpartum mortality than the partial type; hence, prenatal diagnosis is very important.[13] Ultrasound is a very useful method for assessing trisomy 9 mosaicism during the prenatal period. Fetal umbilical cord blood or amniotic cytogenetic testing can be performed if trisomy 9 mosaicism is suspected based on the ultrasound results, but the diagnosis rate is low. When the presence of trisomy 9 cells has been determined, FISH can accurately detect the presence of these cells in metaphase and interphase. In addition, cases of mosaicism have been reported after a repeat FISH of blood and skin fibroblasts was performed in patients diagnosed with non-mosaic type by conventional cytogenetic tests. The majority of the patients with longer than expected survival were diagnosed with mosaic type after performing a repeat FISH.[14]

Since our case showed shortness of breath and low birth weight, delayed intrauterine growth, and craniofacial dysmorphism, we suspected this patient may have chromosomal abnormalities. Thus, an initial peripheral blood chromosome test was performed. A balanced translocation was found from the analysis of 20 dividing cells obtained by culturing from peripheral blood (46, XY, t(2;9)(p22;q33)). Then, we performed an additional microarray analysis to find any microdeletions at the translocation site, but interestingly, the analysis revealed a trisomy 9 mosaicism. When we rechecked 50 dividing cells from peripheral blood after confirming trisomy 9 in the microarray test, we could not find any trisomic cells. An additional FISH test showed 10-15% of trisomy 9 cells among 200 cells. When the fraction of cells with abnormal karyotypes is low, there is a possibility that the abnormal cells will die before detection in cell culture during routine peripheral blood chromosome testing. Therefore, even if the result of the peripheral blood chromosome test is normal, it is recommended that additional microarray tests should be considered if the patient is suspected to have a chromosomal abnormality. The 4 previously reported cases of trisomy 9 mosaicism in

Korea were all confirmed from initial peripheral blood karyotyping test. It is reasonable to consider the possibility that the proportion of abnormal cells in our patient was less than other patients, and that is also the reason for showing a better prognosis than others. The reason for this is that the proportion of cells with a normal karyotype is high and the degree of malformation is relatively mild; hence, patients with this condition may survive longer. Despite the low incidence of trisomy 9 mosaicity, patients with this condition are still at risk of aspiration due to gastroesophageal reflux. Patients who survive longer will likely require further management and education regarding their condition.[15]

We report a case of a male infant diagnosed with trisomy 9 mosaicism who presented with IUGR, facial dysmorphism, and cardiac malformation. Initially, routine peripheral blood karyotype tests did not show any abnormalities; however, the patient was eventually diagnosed with an abnormal finding after performing a cytogenetic microarray test.

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Use of Three-dimensional Transesophageal Echocardiography for the Chiari Network

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The Chiari network is an embryonic remnant of the sinus venosus valve, which is characterized by a fenestrated, net-like structure in the right atrium and has the potential to be misdiagnosed as another right atrial pathology. Additionally, the Chiari network has been frequently reported to entrap intracardiac devices during surgical procedures. In this case report, we present two patients with a Chiari network confirmed by three-dimensional transesophageal echocardiography, which assisted in preventing device entrapment during intracardiac procedures.

Key Words: Chiari network, Swan-Ganz Catheterization, Three-dimensional echocardiography

The Chiari network is characterized by a fenestrated, net-like structure connecting the edges of the inferior vena cava (IVC) and the coronary sinus valve with the crista terminalis.[1] It has the potential to be misdiagnosed as other right atrial (RA) pathologies, such as a tumor, thrombi, vegetations, and Eustachian/Thebesian valves.[2,3] Moreover, several researchers have reported that catheters, guidewires, and pacemaker leads have been entrapped in the Chiari network during intracardiac procedures.[4-6] In recent years, three-dimensional (3D) echocardiography has been developed and its use has spread widely. We report two cases in which 3D transesophageal echocardiography (TEE) was used to confirm a Chiari network diagnosis, which helped to prevent the subsequent entrapment of intracardiac devices during the insertion of central and pulmonary artery catheters.

CASE

Case 1

A 60-year-old man presented to the hospital with aphasia which had started a week previous. A brain magnetic resonance image (MRI) showed a left basal ganglia infarction. The thrombus was suspected to be a fibroelastoma originating in the aortic valve (AV) and this was confirmed by transthoracic echocardiography (TTE). On TTE, a patent foramen ovale (PFO)

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was also identified. He was scheduled for a fibroelastoma resection with PFO closure.

In the operating room, he was intubated and anesthetized with sevoflurane and remifentanil. The left radial artery was cannulated. After insertion of a central venous catheter through the right internal jugular vein, the TEE probe was inserted. We found a linear structure in the RA cavity, which was suspected to be a Chiari network, an Eustachian valve, or a thrombus (Fig. 1A). A 3D TEE image was obtained and a net-like structure was identified, which connected the edge of the IVC to the interatrial septum and was diagnosed as a Chiari network (Fig. 1B). The fibroelastoma on the AV was resected and the PFO was closed successfully.

Case 2

A 60-year-old man presented to the hospital with repeated syncope. His electroencephalography and brain MRI showed normal results. For a more thorough cardiogenic examination, 24-hour Holter monitoring and TTE were performed and the TTE demonstrated severe aortic stenosis due to a bicuspid AV. Moreover, on TTE, whip-like structures extending from the opening of the IVC and moving freely within the RA cavity were observed, which were suspected to be a Chiari network. He was scheduled for AV replacement surgery along with resection of the Chiari network. Because he had a moderate to severe pulmonary hypertension, a pulmonary artery catheter (PAC) insertion was decided.

In the operating room, he was intubated and anesthetized with sevoflurane and remifentanil. The left radial artery was cannulated. Before central venous catheterization, a TEE probe was inserted, and the Chiari network was visualized

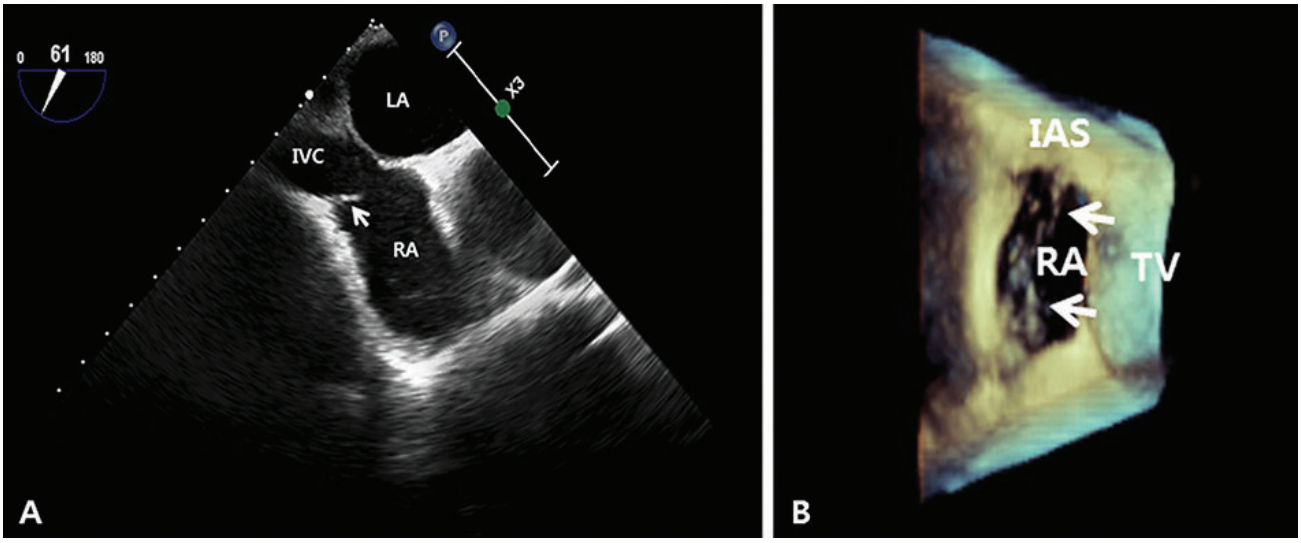

Fig. 1. (A) Two-dimensional transesophageal echocardiography (TEE) showing a linear structure in the right atrium (arrow). (B) Three-dimensional TEE clearly showing a net-like structure connected to the edge of the IVC and interatrial septum. IAS: interatrial septum; IVC: inferior vena cava; LA: left atrium; RA: right atrium; TV: tricuspid valve.

using the midesophageal (ME) modified bicaval tricuspid valve (TV) view focused on the IVC (Fig. 2A). During ultrasound-guided internal jugular venous cannulation, a needle was inserted under the out-plane imaging of the internal jugular vein and a guidewire and catheter were carefully inserted using the ME modified bicaval TV view. Then, the PAC was inserted using the ME modified bicaval TV view, ensuring that the PAC was not entrapped in the Chiari network, in real time. After insertion of the PAC, we scanned the 3D TEE of the RA cavity to confirm that the PAC had passed through the TV without being entrapped in the Chiari network (Fig. 2B). The AV was replaced with a mechanical valve and the Chiari network was resected successfully.

DISCUSSION

The Chiari network is an embryonic remnant of the sinus venosus valve which normally undergoes fenestration and disappears.[7] It is usually found by accident and generally has no major clinical significance. However, sometimes it is difficult to differentiate the Chiari network from other RA pathologies that would require further management. Our first case patient had had a thromboembolic stroke but the origin of the thrombus had not been confirmed. On preoperative TTE, a fibroelastoma was identified on the AV and suspected to be the origin of the thrombus. During intraoperative TEE, we incidentally found a short linear structure which originated from the IVC and was freely moving in the RA

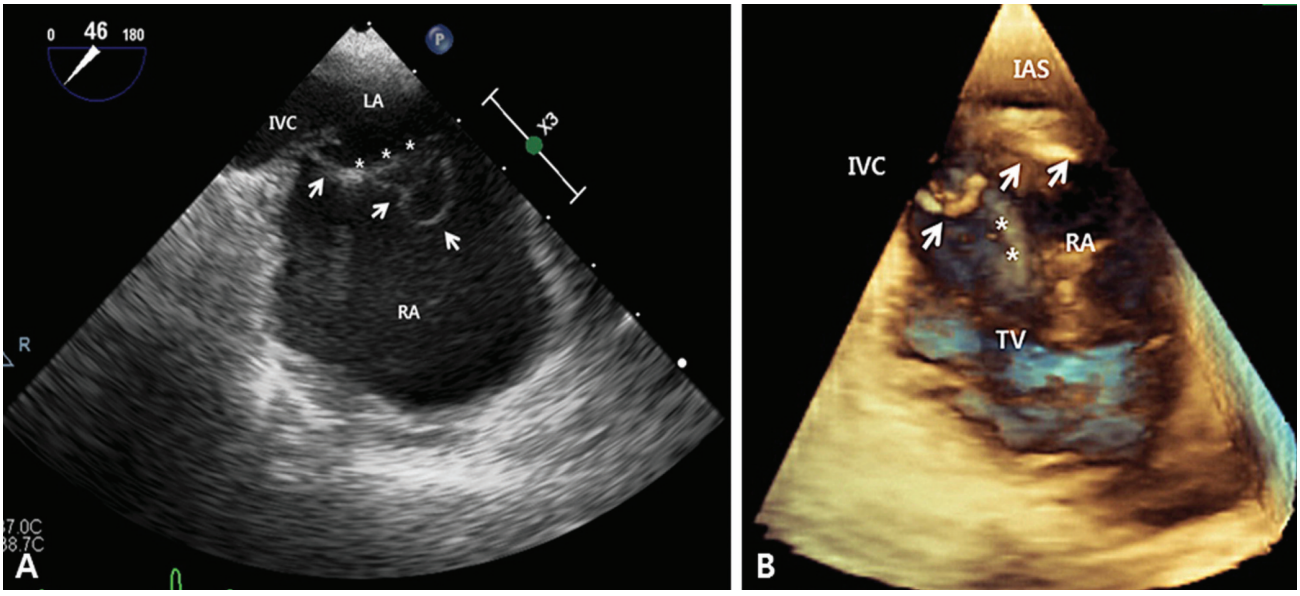

Fig. 2. (A) Two-dimensional transesophageal echocardiography (TEE) imaging does not clearly show the location of the Chiari network (arrow) in relation to the pulmonary artery catheter (PAC, *) (B) Three-dimensional TEE imaging shows that the PAC(*) successfully passed through the tricuspid valve without being entrapped in the Chiari network (arrow). IAS: interatrial septum; IVC: inferior vena cava; LA: left atrium; RA: right atrium; TV: tricuspid valve.

cavity. Considering the patient's history of thromboembolic stroke and PFO, we could not exclude a possible thrombus. On two-dimensional (2D) TEE, it was difficult to differentiate the Chiari network from the thrombus. However, with the 3D TEE view, a net-like structure attached at the edges of the IVC and interatrial septum could be clearly seen.

Intracardiac interventions have recently become more common and several case reports have cautioned that intracardiac devices could be entrapped in the Chiari network.[4-6] In those studies, the entrapped intracardiac devices were removed using several techniques, including simple retraction under echocardiography, placement of a stiff catheter over the trapped guidewire to straighten the device, radiofrequency ablation of the entangled network strands, and even a thoracotomy.[5,8] To prevent the entrapment of intracardiac devices in the Chiari network, real time echocardiography should be used to visualize the Chiari network and the intracardiac devices as they pass through the RA. Therefore, the IVC, the superior vena cava (SVC), the RA, TV, and right ventricle (RV) all should be visualized at the same time. As a result, we decided to observe the insertion of the central catheter and PAC using a ME modified bicaval TV view,[9,10] which can be seen using the ME RV inflow-outflow view and maintaining a transducer angle of 50 to 70 degrees while the probe is turned to the right until the TV is centered.[5] In this case, we needed to focus on the IVC rather than the TV, so the transducer was slightly withdrawn and rotated to the left. The 2D TEE did not clearly show the location of the Chiari network in relation to the PAC (Fig. 2A). However, as Fig. 2B demonstrates, using the 3D TEE view, we could confirm that the PAC passed through the TV without being entrapped in the Chiari network.

Recently, real time 3D TEE guidance during catheter-based interventions have been introduced.[11] However, there are still several limitations to the 3D TEE, such as issues with the frame rate, the spatial resolution, and the 3D color Doppler. Moreover, proficient use requires experience and practice to minimize these problems. During the insertion of catheters in our cases, we could not use the 3D TEE in real time because we lacked this experience and practice. Central catheterization and PAC insertion are not complex and time-consuming catheter-based interventions. If real time 3D TEE guidance during catheter insertion became standard practice, it would provide advantages such as clear visualization of the location of the catheter as it passed through the intracardiac structures and subsequent avoidance of potential complications.

Echocardiography is an excellent diagnostic imaging tool for the Chiari network. However, 2D echocardiography has a limited ability to differentiate structures and to clearly display the location of the Chiari network and intracardiac devices during invasive procedures. We suggest the use of 3D echocardiography when making a definitive diagnosis of Chiari network and to assist in avoiding entrapment and safely inserting a guidewire or catheter during intracardiac procedures.

ACKNOWLEDGEMENTS

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Subcapsular Hepatic Hematoma after Cardiopulmonary Resuscitation

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Cardiopulmonary resuscitation (CPR) is an important life-saving procedure in emergency care. However, CPR is associated with various complications. A 41-year-old man was admitted to the intensive care unit after CPR. A sudden decrease in the blood pressure and hematocrit level was recorded. An abdominal computed tomography (CT) showed a large subcapsular hematoma in the left lobe of the liver. With conservative treatment, the hematoma reduced in size, but it was later managed with percutaneous drainage. The patient recovered and was discharged. We obtained a favorable outcome with conservative, nonsurgical treatment. Subcapsular hepatic hematoma is a potential life-threatening complication that should be considered in CPR survivors.

Key Words: Cardiopulmonary resuscitation, Hematoma, Liver

Cardiopulmonary resuscitation (CPR) is an important, life-saving, emergency care procedure. CPR comprises chest compressions and artificial ventilation; during the former, the lower half of the sternum should be depressed to approximately one-third of the chest cavitary depth in each compression.

Complications of CPR include lung injuries (e.g., pneumothorax), rib fractures and contusion, damage to abdominal organs, and chest and/or abdominal pain.[1-3] However, damage to abdominal organs is a rare complication of CPR. Here, we report a case of subcapsular hepatic hematoma, a rare complication of CPR.[4-6]

CASE

A 41-year-old man was hospitalized with a complaint of muscle weakness that was diagnosed as Guillain–Barres syndrome and appropriately treated. The patient experienced a sudden in-hospital cardiac arrest, was resuscitated, and there was return of spontaneous circulation (ROSC) after 5 minutes of CPR. Subsequently, the patient was admitted in the medical intensive care unit (ICU). Following ROSC, the blood pressure (BP) and heart rate were 105/84 mmHg and 129 beats/min, respectively. The patient was intubated and placed on mechanical ventilation.

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Transthoracic echocardiography revealed the preserved left ventricular function without regional wall motion abnormality. The laboratory tests after CPR showed a white blood cell count of 15.14 × 10^9/L (neutrophils 70.4%), a hemoglobin level of 15.6 g/dL, a platelet count of 282 × 10^3/μL, an aspartate aminotransferase (AST) level of 66 U/L, an alanine aminotransferase (ALT) level of 115 U/L, a blood urea nitrogen level (BUN) of 37.0 mg/dL, pH 7.43, a partial pressure of carbon dioxide of 40 mmHg, a partial pressure of oxygen of 75 mmHg, a bicarbonate concentration of 26.4 mmol/L, and an oxygen saturation of 95.3%. Serum creatinine, albumin, and cardiac enzyme levels; partial thromboplastin time; and prothrombin time were all within normal limits.

On the third day in the ICU, the patient's BP decreased to 87/64 mmHg, and follow-up laboratory tests showed a rapid reduction in the hemoglobin level (7.7 g/dL). Esophagoduodenoscopy did not reveal any active upper gastrointestinal bleeding. Hence, abdominal computed tomography (CT) was performed to identify the source of bleeding, which showed a large subcapsular hematoma (17.8 × 7.9 cm^2) in the left lobe of the liver (Fig. 1). We initially undertook a blood transfusion and conservative, nonsurgical treatment approach and the patient recovered slowly. The hemoglobin level returned to the normal range. There was no increase in the size of the hematoma on follow-up CT (Fig. 2A). In consultation with the surgical team, we inserted a percutaneous drain into the hematoma 1 month after the bleeding was detected (Fig. 2B). After 1 month of PCD insertion, the follow-up CT

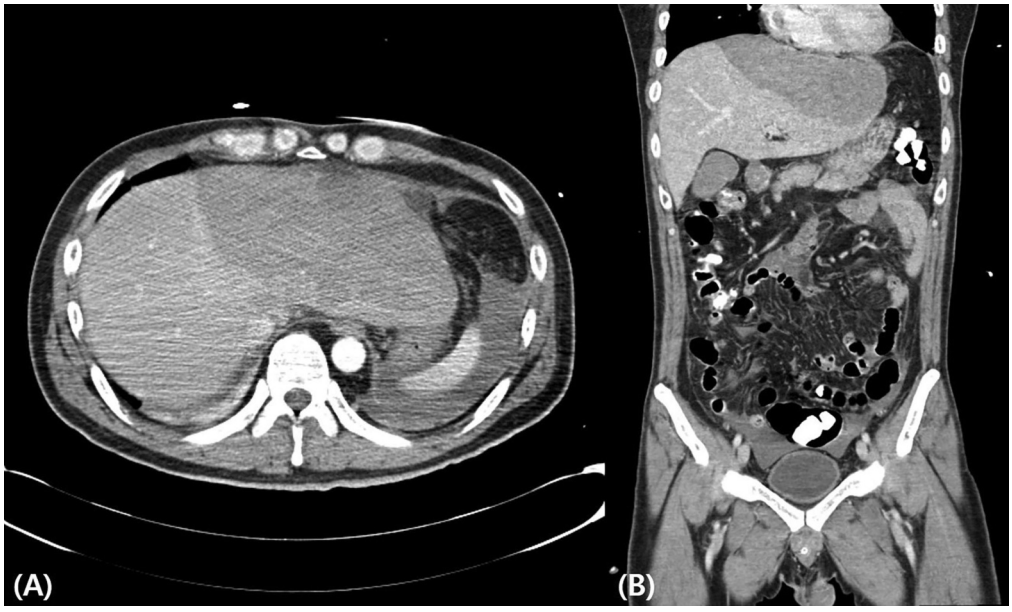

Fig. 1. Abdominal computed tomography showing a large subscapular hematoma (17.8 × 7.9 cm^2) in the left lobe of the liver (A) and small, diluted hemoperitoneum extending to the perisplenic space, mesenteric, and bilateral paracolic gutter and into the pelvic cavity

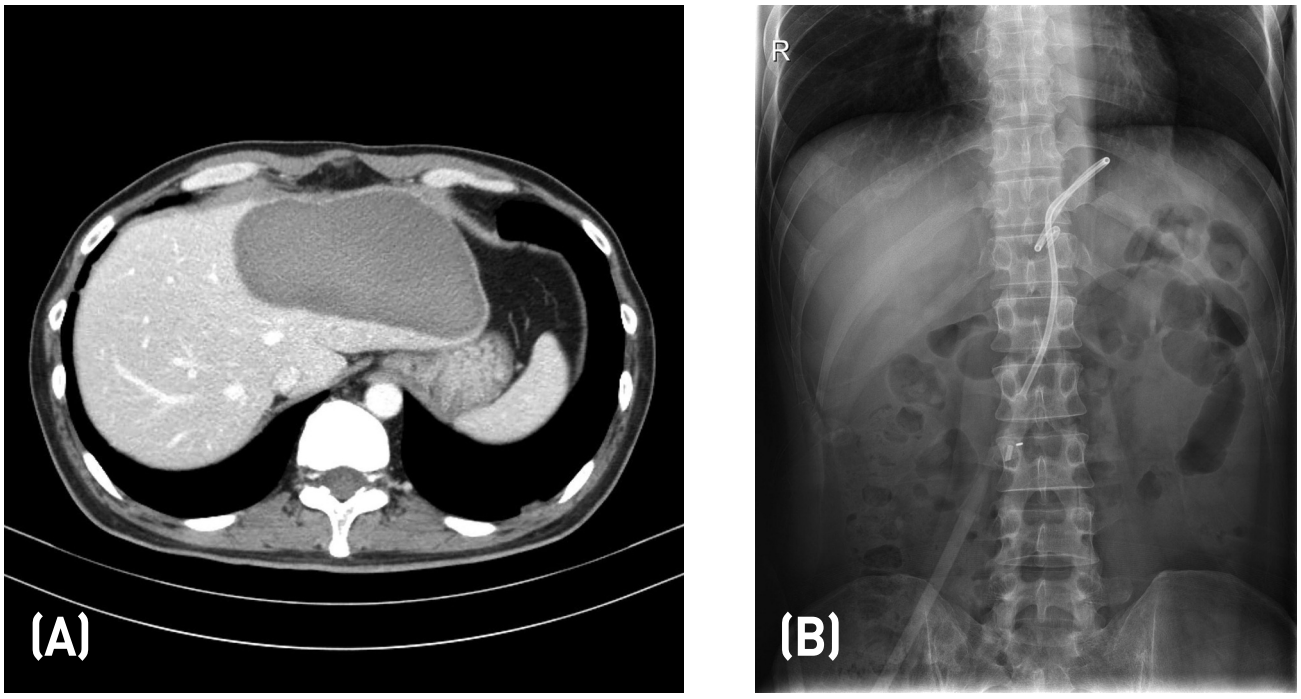

Fig. 2. (A) Follow-up abdominal computed tomography showing subtle decrease in the size of the subscapular hepatic hematoma (14.9 cm from 17.8 cm, in axial size) in the left lobe of the liver and no abnormal fluid collection, (B) Abdominal X-ray showing percutaneous insertion of an abdominal drainage catheter into the subcapsular hepatic hematoma

showed a decrease in hematoma (Fig. 3). Therefore, PCD was removed 1 month after PCD insertion. Subsequently, the patient recovered uneventfully and was discharged.

DISCUSSION

This case report describes a male patient with a subcapsular hepatic hematoma that developed after CPR. For effective CPR, the sternum needs to be depressed 4–5 cm in each compression. Rib fractures are the commonest complication of CPR and can occur in up to 50% of patients.[1-3] Other chest complications include hemothorax, pneumothorax, hemopericardium, and sternal fracture. Intra-abdominal injuries including hepatic, splenic, or intestinal trauma and retroperitoneal hematoma have been reported after CPR.[4,6-8] However, liver injury occurs rarely (0.6%–2%) and is usually associated with anticoagulation therapy.[4,9]

In our case, a subcapsular hepatic hematoma of the left lobe was noted. Liver injury most often occurs in the left lobe during CPR. The most important contributory factor for this is the close anatomical relationship of the left lobe of the liver with lower part of the sternum.[6,10] Hemodynamic instability is a leading symptom of intra-abdominal bleeding. Measuring the hematocrit level might be a useful test in the post-resuscitation phase.[9] When

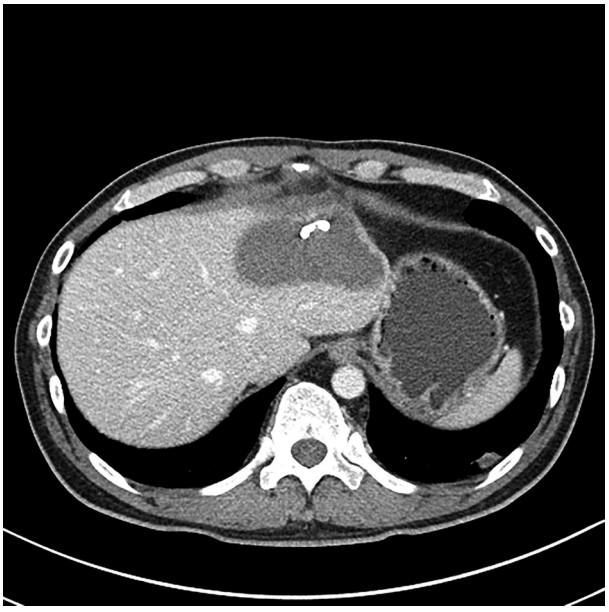

Fig. 3. After 1 month of PCD insertion, the follow-up CT showed decreased in size of large amount of subcapsular hematoma in left lobe of liver (17.8 cm from 9.0 cm, in axial size).

a subcapsular hematoma of the liver is diagnosed, it is important to perform continuous imaging tests and blood tests such as hemoglobin in determining conservative or surgical treatment and to monitor whether the hematoma does not progress.[11] In general, subcapsular hematoma that does not rupture is treated conservatively, and the prognosis is good. In spite of symptomatic treatment, selective embolization of the hepatic artery branch may be considered to control hematoma enlarged or continued bleeding, and octreotide may be used to reduce bleeding.[12] Surgical treatment is recommended for subcapsular hematoma rupture or imminent rupture. Typically, 75% of patients die when the hematoma is ruptured. However, CPR-induced liver injury is usually addressed with conservative management and embolization.[5,6,13] About 7 - 20% of the patients with complications died from the cause of CPCR (eg, cardiac arrest, pulmonary theomboembolism, etc.), but one of them died due to massive bleeding.[4-6,8-10,13-17] Most of the surviving patients recovered without any specific liver-related complications.[4-6,8-10,13-17] In our patient, the vital signs were stable after transfusion, and conservative treatment was recommended on surgical consultation.

Compression during CPR is emphasized with the revision in the resuscitation guideline and is the essential component of basic life support.[18] Frequent interruption of chest compressions does not provide circulatory support for more than half of the resuscitation effort. Such interruptions can be a major cause of the consistent poor results, which manifest as a heart attack.[19] Compression should remain as undisturbed as possible during CPR. With the focus on com-

pression, unusual complications such as a subcapsular hepatic hematoma have been discovered.[5,17] To reduce the occurrence of such complications, it is important to apply compression in the right position with the correct pressure.

In Korea, Wi et al. reported a case of liver laceration with hemoperitoneum after CPR.[6] A 72-year-old female patient developed severe hemoperitoneum associated with liver injury after CPR and underwent emergency embolization, but she died.[6] In addition, in Korea, there have been cases of spontaneous liver injury occurring after delivery,[20] after endoscopic retrograde cholangiopancreatography,[21] during hemodialysis,[22] liver abscess,[23] idiopathic[24] and recovery with conservative treatment in most cases. However, in our case, conservative treatment alone recovered liver injury following CPR.

In patients with a sudden drop in hematocrit level and blood pressure after CPR, it is important to evaluate the hemorrhagic foci. Despite liver injury being an uncommon finding in such an evaluation, it is important to consider it in the differential diagnosis to facilitate correct diagnosis and treatment, which will improve the patient's prognosis.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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3) Book with a single author

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

4) Chapter in a multiauthored book with editors

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.

6) Internet or electronic resource

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

American Medical Association; c1995-2007 [cited 2007 Feb 22]. Available from: http://www.ama-assn.org/.

7) Others

All other references should be follow the Vancouver style (N Engl J Med 1997;336:309-15).

8) DOI

Plese remove the "[pii]" field in any doi. E.g., doi:00932.2006 [pii] 10.1152/japplphysiol.00932.2006. Remove "00932.2006 [pii]".

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