ISSN 2187-8072

NINGEN DOCK INTERNATIONAL

Official Journal of Japan Society of Ningen Dock

JAPAN SOCIETY OF NINGEN DOCK

Vol.6 No.1
2019. 3

Ningen Dock International

Official Journal of Japan Society of Ningen Dock

Editorial Board

Published by Japan Society of Ningen Dock
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Ningen Dock International is the official English-language journal of Japan Society of Ningen Dock.
Business matters should be addressed to Japan Society of Ningen Dock.

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Relationship of Atherosclerotic Risk Factors with Pulmonary Age and Vascular Age

Masao Shimizu, Asako Okano, Masaki Adachi, Yoshiaki Maruyama

Abstract

Background: Different organs undergo different atherosclerotic changes due to aging. This study examined the effects of atherosclerotic risk factors on pulmonary and vascular age.

Methods: Our subjects were 531 persons whose pulmonary and vascular age was measured at our center. First, a correlation between pulmonary age and cardio-ankle vascular index (CAVI), an indicator of vascular age, was examined. Based on mean pulmonary age (60.8) and CAVI (8.53), subjects were divided into four groups (G I: CAVI ≤ 8.5 pulmonary age ≤ 60, G II: CAVI ≥ 8.6 pulmonary age ≤ 60, G III: CAVI ≤ 8.5 pulmonary age ≥ 61, G IV: CAVI ≥ 8.6 pulmonary age ≥ 61). Groups were compared in terms of chronological age, sex, affected atherosclerotic disease (high blood pressure (HBP), dyslipidemia, diabetes mellitus (DM)), blood pressure, low-density lipoprotein cholesterol, triglyceride, high-density lipoprotein cholesterol, fasting blood sugar, HbA1c, waist circumference, bone density, aortic calcification score and smoking score.

Results: A moderate, positive correlation was seen between pulmonary age and CAVI. Comparisons with atherosclerotic risk factors in the four groups revealed: i) HbA1c was associated with increased pulmonary age; ii) diastolic blood pressure and aortic calcification score were associated with increased CAVI; and iii) HBP, dyslipidemia, chronological age and systolic blood pressure were associated with increases in both pulmonary age and CAVI.

Conclusion: Although a correlation was seen between pulmonary and vascular age, it was not particularly high. The present study revealed that this was because atherosclerotic risk factors affect pulmonary and vascular age differently.

Keywords atherosclerotic risk factors, pulmonary age, vascular age, CAVI

Chronic obstructive pulmonary disease (COPD) is a systemic disease[1], and a growing number of studies have reported associations between COPD and atherosclerosis[2–4].

Pulmonary function tests are gaining attention due to the increasing number of patients with COPD[5]. Of all the pulmonary function tests, forced expiratory volume in 1 s (FEV1) shows the best correlation with chronological age[5], and the Japanese Respiratory Society has proposed the use of pulmonary age as an indicator of the degree of aging of the lungs[6]. Arterial stiffness is correlated with risk of cardiovascular lesions[7], and the cardio-ankle vascular index (CAVI) has been drawing attention as a new indicator of arterial stiffness (vascular age) because it is a convenient, non-invasive measure that is independent of blood pressure[8–10].

However, according to studies that have investigated correlations between pulmonary age and CAVI, the degree of correlation is not particularly high[11]. We hypothesized that atherosclerotic risk factors may affect pulmonary and vascular age differently and to verify it, examined the effects of atherosclerotic risk factors on pulmonary age and vascular age.

Methods

Subjects

This study recruited 531 consecutive subjects whose pulmonary age and CAVI were measured at our center between September 1, 2016 and May 31, 2017. Approval for this study was obtained from the Saitama Medical University Hospital Ethics Committee and informed consent was obtained from all participants.

Measurements

The Pulmonary age and CAVI of all subjects were first measured and a correlation was examined. Based on mean pulmonary age (60.8) and CAVI (8.53), subjects were divided into four groups (G I: CAVI ≤ 8.5 pulmonary age ≤ 60, G II: CAVI ≥ 8.6 pulmonary

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age ≤ 60, G III: CAVI ≤ 8.5 pulmonary age ≥ 61, G IV:CAVI ≥ 8.6 pulmonary age ≥ 61), and the groups were compared in terms of qualitative and quantitative atherosclerotic risk factors. Qualitative variables were sex breakdown and affected atherosclerotic disease (high blood pressure (HBP), dyslipidemia, diabetes mellitus (DM)). Quantitative variables were chronological age, waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), pulse pressure (PP), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), fasting blood sugar (FBS), hemoglobin A1c (HbA1c), bone density, aortic calcification score (Ca score) and smoking score. Ca score was determined based on the number of calcified aorta at the ascending aorta, aortic arch, chest descending aorta and abdominal descending aorta, from 0 to 4. The smoking score was 0 for not smoking, 1 for quit smoking and 2 for still smoking.

Devices used were: i) a spirometer for pulmonary age (Spirosift SP-7710; Fukuda Denshi, Tokyo, Japan); and ii) a vascular screening system for CAVI (VaSera VS-1500N; Fukuda Denshi, Tokyo, Japan).

Statistical analysis

Proportional comparisons among the four groups for qualitative variables were performed using the chi-square test, and this was followed by the Z-test for statistically significant variables. Quantitative variables were expressed as mean ± s.d., and differences among the four groups were compared by one-way analysis of variance (ANOVA), followed by the Bonferroni post hoc test. Values of $p < 0.05$ were considered statistically significant for the chi-square test, ANOVA and Z-test, and values of $p < 0.0083$ were considered significant for the Bonferroni test. SPSS version 24 (IBM, Chicago, IL, USA) was used for the statistical analysis.

Results

Table 1 shows the sex difference between measured qualitative and quantitative variables, and results of comparison.

Correlation between pulmonary age and CAVI

A significant, moderate, positive correlation was evident between pulmonary age and CAVI (pulmonary age = 6.99 × CAVI+1.22 r=0.445 ($p < 0.001$)) (**Fig. 1**).

Comparison of atherosclerotic risk factors among four groups

Table 2 shows the results of the chi-square test for qualitative variables and ANOVA for quantitative variables. Significant differences among the four groups were observed in the following atherosclerotic risk factors: i) qualitative variables (sex breakdown, HBP, dyslipidemia and DM); ii) quantitative variables (chronological age, waist circumference, SBP, DBP, MBP, PP, HDL-C, FBS, HbA1c, Ca score and Smoking score). These factors were used in the next analysis.

Fig. 2-1 and **Fig. 2-2** show the results of the Z-test for qualitative variables and Bonferroni test for quantitative

Table 1. Characteristics of Study Subjects

	Men	Women	*p*
n (%)	321 (60.5%)	210 (39.5%)	<0.001
HBP (%)	123 (38.3%)	49 (23.3%)	0.001
Dyslipidemia (%)	104 (32.4%)	50 (23.8%)	ns
DM (%)	49 (15.3%)	6 (2.9%)	<0.001
Chronological age (years)	63.3 ± 9.9	63.1 ± 3.8	ns
Waist circumference (cm)	85.7 ± 8.4	80.1 ± 8.3	<0.001
SBP (mmHg)	126.5 ± 13.5	120.5 ± 15.0	<0.001
DBP (mmHg)	78.5 ± 9.1	73.9 ± 9.6	<0.001
MBP (mmHg)	110.5 ± 11.4	104.9 ± 12.6	<0.001
PP (mmHg)	48.0 ± 9.0	46.6 ± 10.3	ns
LDL-C (mg/dL)	122.8 ± 29.8	73.9 ± 9.7	0.023
TG (mg/dL)	117.1 ± 76.9	92.7 ± 43.6	<0.001
HDL-C (mg/dL)	58.9 ± 14.5	72.5 ± 16.9	<0.001
FBS (mg/dL)	106.6 ± 21.2	98.0 ± 11.3	<0.001
HbA1c (%)	5.7 ± 0.7	5.5 ± 0.3	<0.001
Bone density (g/cm^2)	0.60 ± 0.09	0.44 ± 0.09	<0.001
Ca score	1.96 ± 1.34	1.67 ± 1.38	ns
Smoking score	0.78 ± 0.67	0.20 ± 0.53	<0.001
Pulmonary age (years)	65.0 ± 18.5	54.4 ± 16.1	<0.001
CAVI	8.8 ± 1.1	8.2 ± 1.1	<0.001

Variables are given as number (%) or mean ± s.d.
HBP: high blood pressure, DM: diabetes mellitus, SBP: systolic blood pressure, DBP: diastolic blood pressure, MBP: mean blood pressure, PP: pulse pressure, LDL-C: low-density lipoprotein cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, FBS: fasting blood sugar

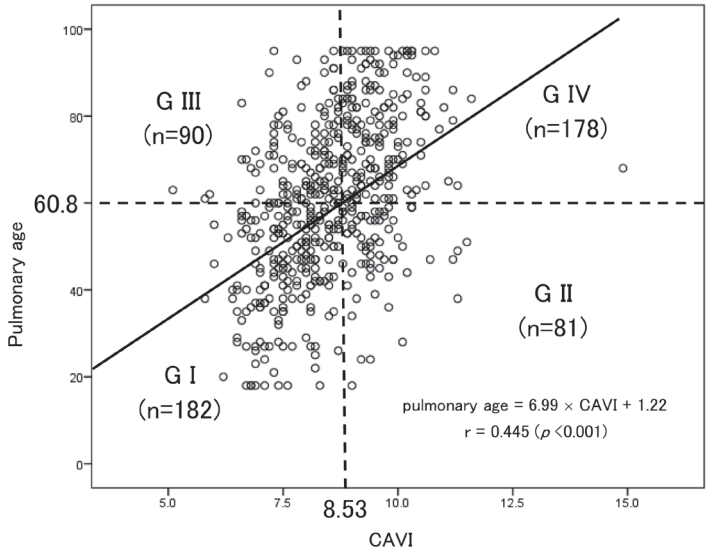

Fig.1. Correlation of Pulmonary Age and CAVI in Subjects Divided into 4 Groups by Mean of Pulmonary Age and CAVI
60.8: mean of pulmonary age; 8.53: mean of CAVI. A significant, moderate, positive correlation was evident between pulmonary age and CAVI (pulmonary age = 6.99 × CAVI+1.22 r=0.445 (p <0.001)). Subjects were divided into four groups based on the means of the parameters (pulmonary age: 60.8 years; CAVI: 8.53).

Table 2. Comparison of Atherosclerotic Risk Factors Among Four Groups

	G I (*n*=182)	G II (*n*=81)	G III (*n*=90)	G IV (*n*=178)	*p*
Sex breakdown: Men (%)	79 (43.4%)	47 (58.0%)	57 (63.3%)	138 (77.5%)	<0.001
HBP (%)	24 (13.2%)	27 (33.3%)	34 (37.8%)	88 (49.4%)	<0.001
Dyslipidemia (%)	33 (18.1%)	29 (35.8%)	31 (34.4%)	62 (34.8%)	<0.006
DM (%)	6 (3.3%)	7 (8.6%)	10 (11.1%)	33 (18.5%)	<0.001
Chronological age (years)	55.7 ± 8.8	66.0 ± 6.1	62.6 ± 9.1	70.0 ± 6.7	<0.001
Waist circumference (cm)	81.6 ± 8.6	83.8 ± 6.8	84.7 ± 10.7	84.7 ± 8.3	0.003
SBP (mmHg)	117.8 ± 13.9	128.3 ± 12.7	126.0 ± 14.2	127.6 ± 13.5	<0.001
DBP (mmHg)	74.3 ± 10.2	78.4 ± 8.4	78.1 ± 8.7	77.6 ± 9.4	0.001
MBP (mmHg)	103.3 ± 12.3	111.7 ± 10.2	110.0 ± 11.8	110.9 ± 11.4	<0.001
PP (mmHg)	43.5 ± 7.4	49.9 ± 10.9	47.9 ± 9.5	50.1 ± 9.7	<0.001
LDL-C (mg/dL)	126.6 ± 28.6	129.4 ± 29.9	126.3 ± 31.4	121.0 ± 28.2	ns
TG (mg/dL)	104.4 ± 91.2	108.5 ± 39.0	111.8 ± 61.8	107.9 ± 47.1	ns
HDL-C (mg/dL)	68.5 ± 18.3	63.0 ± 15.4	62.7 ± 15.3	61.3 ± 16.0	<0.001
FBS (mg/dL)	98.7 ± 18.3	101.6 ± 10.5	105.2 ± 21.4	107.5 ± 18.7	<0.001
HbA1c (%)	5.5 ± 0.5	5.5 ± 0.3	5.7 ± 0.7	5.8 ± 0.6	<0.001
Bone density (g/cm^2)	0.52 ± 0.10	0.44 ± 0.11	0.54 ± 0.15	0.51 ± 0.12	ns
Ca score	1.2 ± 1.2	1.9 ± 1.3	1.3 ± 1.2	2.6 ± 1.2	<0.001
Smoking score	0.54 ± 0.74	0.37 ± 0.56	0.52 ± 0.62	0.66 ± 0.67	0.016

Variables are given as number (%) or mean ± s.d.
HBP: high blood pressure, DM: diabetes mellitus, SBP: systolic blood pressure, DBP: diastolic blood pressure, MBP: mean blood pressure, PP: pulse pressure, LDL-C: low-density lipoprotein cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, FBS: fasting blood sugar

variables, respectively.

In terms of sex breakdown, men predominated in the high pulmonary age group (G I vs G III $p < 0.05$, G II vs. G IV $p < 0.05$). HbA1c was significantly higher in G IV than in G II ($p = 0.008$). DBP was significantly higher in G II than in G I ($p = 0.007$), and a significant difference in Ca scores was evident between G III and G IV ($p < 0.001$). HBP (G II vs. G III $p < 0.05$, G I vs. G III $p < 0.05$), dyslipidemia (G I vs. G II $p < 0.05$, G I vs. G III $p < 0.05$), chronological age (G I vs. G II $p < 0.001$, G I vs. G III p < 0.001), SBP (G I vs. G II $p < 0.001$, G I vs. G III $p < 0.001$), MBP (G I vs. G II $p < 0.001$, G I vs. G III $p < 0.001$) and PP (G I vs. G II $p < 0.001$, G I vs. G III $p < 0.001$) were significantly higher in G II and G III than in G I.

Discussion

Decreased pulmonary function and arterial stiffness are factors associated with atherosclerosis with a common background[12]. Both COPD and CAVI have associations with atherosclerosis and studies have reported the involvement of inflammatory responses and

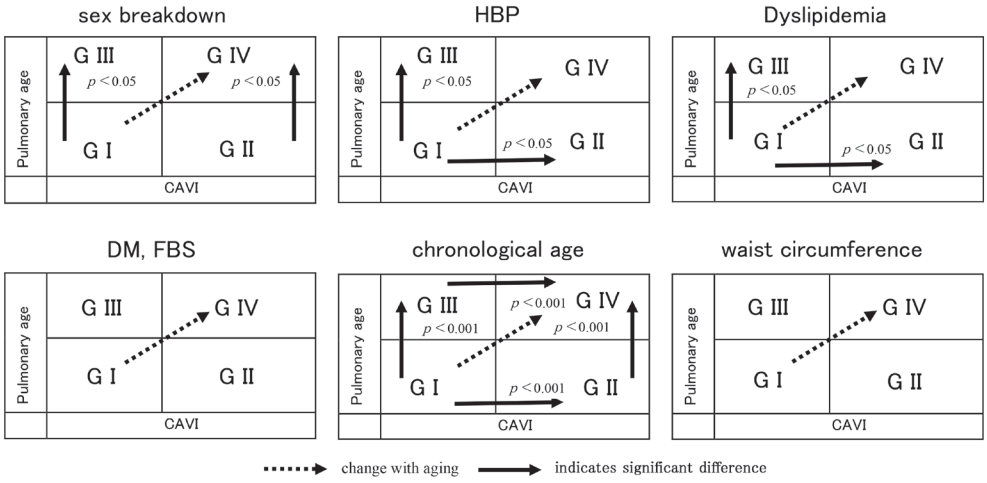

Fig.2-1. Comparison of Atherosclerotic Risk Factors Among Four Groups

Comparison of sex breakdown, HBP, Dyslipidemia, DM, FBS, chronological age and waist circumference among the four groups. In terms of sex breakdown, men predominated in the high pulmonary age groups. HBP, dyslipidemia and chronological age were significantly higher in G II and G III than in G I.

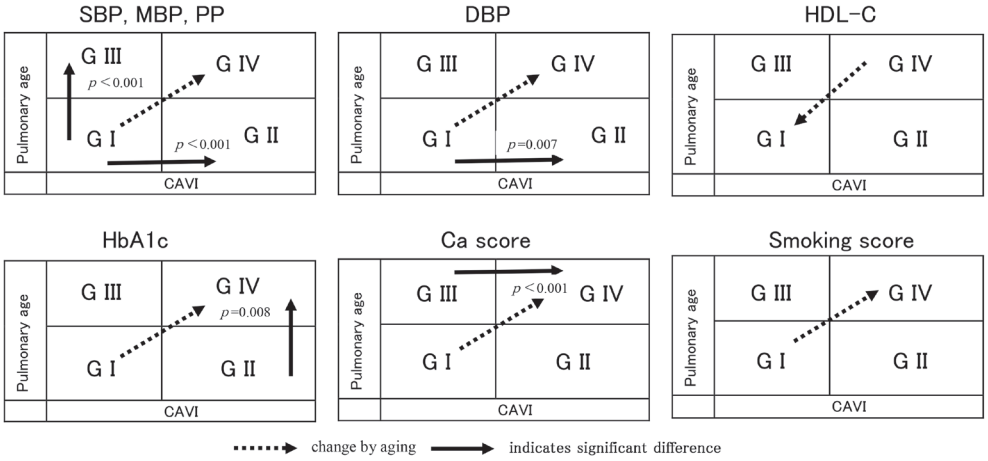

Fig.2-2. Comparison of Atherosclerotic Risk Factors Among Four Groups

Comparison of SBP, MBP, PP, DBP, HDL-C, HbA1c, Ca score and Smoking score among the four groups. HbA1c was significantly higher in G IV than in G II. DBP was significantly higher in G II than in G I. Ca score was significantly higher in G IV than in G III. SBP, MBP and PP were significantly higher in G II and G III than in G I.

high-sensitivity CRP (hs-CRP) as common factors[13–15]. However, in a study examining the degree of correlation between pulmonary age and CAVI in hypertensive patients, the correlation coefficient was not particularly high (r = 0.559)[5], similarly to our finding (r = 0.445). One reason may be that atherosclerotic risk factors affect pulmonary and vascular age differently.

Factors increasing pulmonary age

This study showed that HbA1c was a factor associated with increased pulmonary age. Previous studies have shown that HbA1c is an exacerbating factor for COPD[16,17], but mechanisms remain unclear. Since HbA1c exhibits seasonal variation[18], this may be related to seasonal exacerbations in COPD patients. Further studies are clearly warranted.

Factors increasing CAVI

The present study found that DBP and Ca score were factors associated with increased CAVI. In general, DBP increases with age until about 50 years old, and thereafter likely declines as PP increases[19]. In our study, a significant increase in DBP was seen in G II. The mean age in G I was 55.7 ± 8.8 years, compared to 66.0 ± 6.1 years in G II. The mean age of G II was significantly higher than that of G III (62.6 ± 9.1 years), but in G II, DBP had not declined despite the high age. In other words, peripheral vascular resistance had increased more than age and this was believed to be involved in the increase in CAVI.

Chronological age, HBP, dyslipidemia, DM and smoking are factors known to be associated with aortic calcification[20]. Also, increased afterload from an elevated

central aortic blood pressure due to wave reflection is thought to be involved in the calcification of the aortic arch, rather than systemic blood pressure[21]. An increase in afterload may be associated with an increase in CAVI, but the mechanisms underlying the development of aortic calcification may vary depending on the site.

Factors that increase both pulmonary age and CAVI

This study identified HBP, SBP, MBP, PP and dyslipidemia as factors associated with increases in both pulmonary age and CAVI.

In a study of patients with hypertension, 20% of them had COPD as a complication and approximately 30% of COPD patients had hypertension[22]. In other words, hypertension appears to be a factor associated with increased pulmonary age. While CAVI is unaffected by blood pressure, patients with hypertension are known to exhibit a high CAVI[23], and hypertension is thus a factor associated with increased CAVI.

Lipid-lowering therapy with statins is effective not only in heart failure, but also in COPD[24], and dyslipidemia is thus believed to be a factor associated with increased prevalence of COPD. Dyslipidemia is almost certainly a factor that increases CAVI, since a positive correlation has been observed between serum lipid levels and CAVI in patients with hypertension[25], and CAVI is high in dyslipidemia patients[26].

Study limitations

The sex breakdown in this study may have affected our results. Men tended to have higher levels for many atherosclerotic risk factors other than chronological age (**Table 1**). In future studies, we will need to increase the number of subjects to improve examinations in terms of sex. The association of pulmonary age and smoking score were slightly short ($p = 0.009$) of the level of significance. There may have been a problem in how the smoking score was defined, and this will have to be looked at in future studies.

Conclusions

Although a correlation was seen between pulmonary and vascular age, it was not particularly high. One plausible explanation is that atherosclerotic risk factors may have different effects on pulmonary and vascular age.

This study was presented orally at the plenary session of the 58th Scientific Meeting of the Japanese Society of Ningen Dock (August 2017 Omiya).

Conflict of Interest

There are no conflicts of interest to declare concerning this study.

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(Received February 15, 2018 ; Accepted April 24, 2018)

Original Article

Ningen Dock International 2019 ; 6 : 9–17

Association of Coffee Intake and Other Factors with Sleep Duration and Sleep Satisfaction: Results of Analysis of Large Cross-sectional Study

Takeshi Shimamoto[1,2], Nobutake Yamamichi[1], Ryoichi Wada[2], Toru Mitsushima[2], Kazuhiko Koike[1]

Abstract

Objective: The purpose of this study was to perform a detailed statistical analysis on coffee's effect on sleep in Japan from an epidemiological perspective.

Methods: Subjects were 9,839 men (mean age, 50.7 ± 9.2 years) and 7,148 women (mean age, 49.4 ± 8.9 years) who received a health check-up. Coffee intake, total sleep duration, and sleep satisfaction were investigated and evaluated using univariate and multivariate analyses.

Results: Our results are for: Coffee intake [<1 cup/day: *n*=5,430 (32.0%), 1 cup/day: *n*=2,540 (14.9%), 2 cups/day: *n*=3,647 (21.5%), and ≥3 cups/day: *n*=5,370 (31.6%)], total sleep duration [average 6.1± 0.95 h/day; breakdown, <5 h/day: *n*=511 (3.0%), 5–7 h/day: *n*=10,842 (63.8%), 7–9 h/day: *n*=5,555 (32.7%), and ≥9 h/day: *n*=79 (0.5%)], sleep satisfaction [satisfaction: *n*=10,030 (59.0%), and dissatisfaction: *n*=6,957 (41.0%)]. Total sleep duration and sleep satisfaction increased with age, and were both poorer for women than men. Subjects satisfied with their sleep had a significantly longer sleep duration than those who did not (6.5 ± 0.85 h vs. 5.6 ± 0.82 h). Multivariate analyses showed that male gender, older age, smoking, drinking, and preferable dietary/fitness habits had a significantly positive association with both total sleep duration and sleep satisfaction.

Conclusions: Coffee had a weak but significant association with total sleep duration in subjects who drank ≥2 cups/day. On the other hand, no association was observed between coffee intake and sleep satisfaction.

Keywords sleep duration, sleep satisfaction, coffee intake

Sleep plays an important role in human physical health. Continued sleep deprivation increases the risk of several chronic health problems and among the physiological effects of sleep deprivation previously reported are increased risk of obesity[1–4], diabetes[5–9], hypertension[10,11], abnormal hormone levels[12–16], and cardiovascular disease[17–20]. Recent studies have also reported that sleep deprivation can damage brain cells[21] and also cause deposition of amyloid-β protein, which leads to memory disorders and Alzheimer's disease[22]. Another study has revealed that sleep has a restorative function based on the detoxification of potentially neurotoxic waste products[23].

Among many countries worldwide, sleep duration in Japan is one of the shortest[24]. Also according to a recent survey that investigated the current status of sleep in Japan, nearly 30% of Japanese have sleep disorders[25]. Sleep deprivation is a problem of public health that cannot be ignored, especially in developed countries, where a wide variety of social factors and lifestyles can cause sleep disorders. Therefore, we are of the opinion that detailed investigation of factors that may inhibit sleep is important.

Coffee is a factor that possibly influences sleep because its stimulatory effect on sympathetic nerves inhibits the function of sleep[26–32]. Furthermore, drinking a cup of coffee before bedtime could affect the sleep cycle[33]. Coffee is one of the most widely consumed beverages in the world, and Japan is one of the biggest coffee markets in Asia[34]. Therefore, in the present study, we aimed to evaluate the possible effects of coffee intake on sleep in consideration of age, gender, dietary habits and fitness habits.

In summary, the purpose of this study was to perform a detailed statistical analysis, from an epidemiological perspective, of the effect of coffee on sleep.

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Using large amounts of data from healthy subjects, we investigated the current status of sleep and coffee intake in Japan and also examined associations between sleep status and background factors including age, gender, dietary habits, fitness habits and coffee intake.

Methods

Study Subjects

The study subjects were 16,987 adults with no missing data who underwent a health check-up at Kameda Medical Center in Makuhari from January 4 to December 28, 2010. The gender breakdown of participants was 9,839 men (mean age 50.7 ± 9.2 years, range 19–86 years) and 7,148 women (mean age 49.4 ± 8.9 years, range 20–87 years). This study was approved by the ethics committees of the University of Tokyo, and written informed consent was obtained from each subject before study participation in accordance with the Declaration of Helsinki.

Questionnaires

The Ministry of Health, Labour and Welfare of Japan established the system for Specific Health Examinations and Specific Health Guidance based on scientific evidence in 2007 and it went into operation in fiscal 2008 [35,36]. For our research, we used some parts of the questionnaires that are used under the medical care system. Regarding fitness habits, we asked the following questions: "Have you been doing exercise that produces light sweating for over 30 minutes a time, twice weekly, for over a year ?" and "In your daily life, do you walk or engage in an equivalent amount of physical activity more than one hour a day ?". We also surveyed dietary habits, asking: "Is your speed of eating quicker than that of others ?", "Do you eat supper 2 hours before bedtime more than 3 times a week ?", "Do you eat snacks after supper more than 3 times a week ?" and "Do you skip breakfast more than 3 times a week ?".

In addition to the above, we analyzed answers to the following 5 questions: i) "How often do you drink alcohol in a week ?"; ii) "Do you have a smoking habit?"; iii) "Do you sleep well and enough ?"; iv) "How long do you sleep every day ?"; and v) "How much coffee do you drink ?". The answer to question i) was selected from five options (never, seldom, sometimes, often, and always), which were used as nominal variables to categorize subjects into 2 groups: rarely drinking group (never or seldom) and habitually drinking group (sometimes, often, or always). The answers to question ii) were used as nominal variables to categorize subjects into two groups: current or past habitual smoking (smoker group), and lifelong nonsmoking (nonsmoker group). The answer to iii) was "yes" or "no". The answers to question iv) used default values. The answers to question v) were used as ordinal variables to categorize subjects into three groups: drinking less than a cup of coffee per day, 1–2 cups of coffee per day, and 3 or more cups of coffee per day. Answers provided by the participants were carefully checked by nursing staff before being recorded in our study database.

Statistical analysis

We used JMP 13.2.1 or SAS Universal Edition (SAS Institute Inc. Cary, NC, USA) for statistical analysis. Effect size (ES) indicating the quantitative measure of the intensity of the phenomenon was calculated using G*power[37]. In the univariate analysis by age and gender, to evaluate associations between coffee intake and total sleep duration or sleep satisfaction, we used Welch's test, the chi-square test, Jonckheere-Terpstra trend test, Cochran-Armitage trend test and Kruskal-Wallis test, one-way analysis of variance; for multivariate analysis, we used multiple linear regression analysis and multiple logistic regression analysis. Total sleep duration and sleep satisfaction were defined as predictive factors, and age, gender, coffee, a cigarette, an alcoholic beverage, fitness habits, dietary habit, and adult disease (diabetes, obesity, hypertension, and dyslipidemia) were defined as background factors. In all analyses, two-tailed p values < 0.05 were considered statistically significant.

Results

Characteristics of coffee intake, sleep duration and sleep satisfaction in subjects

The characteristics of coffee intake and sleep status in the study group were as follows. About 68.0% of the subjects overall (68.4% for men and 67.5% for women) drank one or more cups of coffee per day. Subjects in the 40–49 age group drank the most coffee, for both men and women. Coffee intake differed between the genders as well as among the six age groups, with both statistical ($p < 0.001$) and practical significance (ES = 0.15–0.16) (**Fig. 1**). The total sleep duration of women was significantly shorter than that of men ($p < 0.001$). Total sleep duration increased with age but regardless of gender ($p < 0.001$ by Jonckheere–Terpstra trend test) (**Table 1**). The rate of sleep satisfaction was significantly different between the two genders and among the six age groups ($p < 0.001$ by chi-square test). With regard to gender, the percentage of women who were satisfied with their sleep was much smaller than that of men (53.8% vs. 62.9%). With regard to age, subjects in the 40–49 age group were most frequently dissatisfied with their sleep. Nevertheless, the Cochran–Armitage trend test revealed that the degree of sleep satisfaction followed an increasing trend proportionally with age for both men and women ($p < 0.001$ for both) (**Fig. 2**).

Associations of coffee intake with total sleep duration and sleep satisfaction in univariate analyses

The associations of coffee intake with total sleep du-

(a)

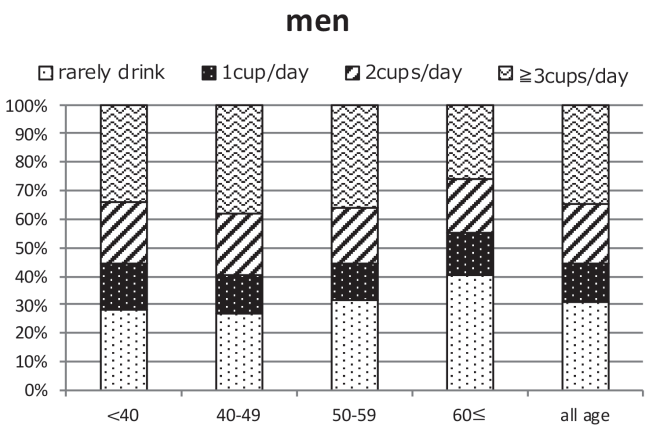
age group	men				
	rarely drink	1cup /day	2cups /day	≧3cups /day	overall
<40	330 (28.5)	182 (15.7)	252 (21.8)	394 (34.0)	1158 [11.8]
40-49	877 (26.8)	443 (13.6)	717 (21.9)	1232 (37.7)	3269 [33.2]
50-59	1200 (31.9)	464 (12.3)	750 (20.0)	1346 (35.8)	3760 [38.2]
60≤	701 (42.4)	253 (15.3)	299 (18.1)	399 (24.2)	1652 [16.8]
all ages	3108 (31.6)	1342 (13.6)	2018 (20.5)	3371 (34.3)	9839 [100.0]
P value :		Chi-squared test			<0.001
effect size :		Cohen's *w*			0.16

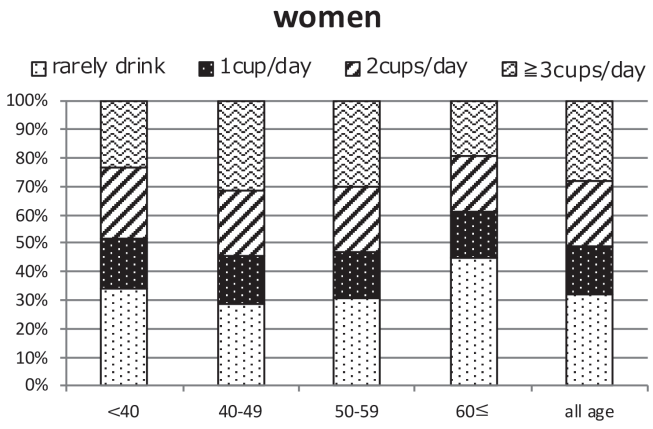
(b)

age group	women				
	rarely drink	1cup /day	2cups /day	≧3cups /day	overall
<40	343 (33.9)	179 (17.7)	253 (25.0)	236 (23.3)	1011 [14.1]
40-49	735 (28.5)	437 (16.9)	601 (23.3)	807 (31.3)	2580 [36.1]
50-59	821 (30.9)	434 (16.3)	609 (22.9)	794 (29.9)	2658 [37.2]
60≤	423 (47.0)	148 (16.5)	166 (18.5)	162 (18.0)	899 [12.6]
all ages	2322 (32.5)	1198 (16.7)	1629 (22.8)	1999 (28.0)	7148 [100.0]
P value :		Chi-squared test			<0.001
effect size :		Cohen's *w*			0.16

(c)

age group	overall				
	rarely drink	1cup /day	2cups /day	≧3cups /day	overall
<40	673 (31.0)	361 (16.6)	505 (23.3)	630 (29.1)	2169 [12.8]
40-49	1612 (27.5)	880 (15.1)	1318 (22.5)	2039 (34.9)	5849 [34.4]
50-59	2021 (31.5)	898 (14.0)	1359 (21.2)	2140 (33.3)	6418 [37.8]
60≤	1124 (44.1)	401 (15.7)	465 (18.2)	561 (22.0)	2551 [15.0]
all ages	5430 (32.0)	2540 (15.0)	3647 (21.4)	5370 (31.6)	16987 [100.0]
P value :		Chi-squared test			<0.001
effect size :		Cohen's *w*			0.15





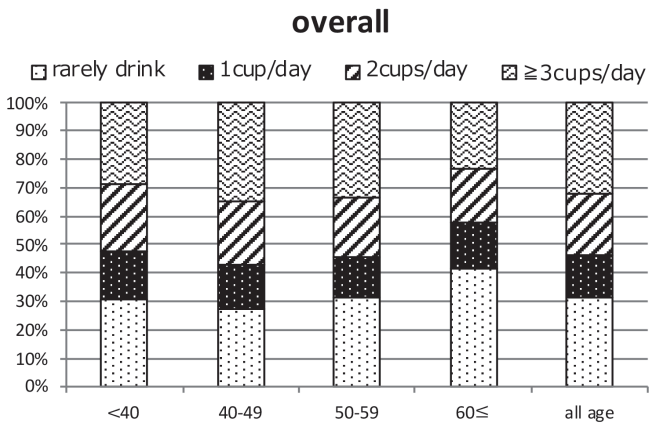


Fig.1. Coffee Intake and Age Group in Men, Women, and Subjects Overall

All panels show a frequency distribution table on the left, and a histogram 100% stacked column chart on the right. Panel (a) shows data for men, panel (b) shows data for women, and panel (c) shows data for both genders together. Values in ( ) in the frequency distribution table are percentages for rows, and values in [ ] are percentages for columns.

ration and sleep satisfaction are shown in **Table 2**. The Jonckheere–Terpstra trend test revealed that both total sleep duration and sleep satisfaction decreased with increase in coffee intake ($p < 0.001$). Subjects in the sleep satisfaction group had a longer sleep duration compared with subjects in the sleep dissatisfaction group, and the difference was statistically (6.5 ± 0.85 h vs. 5.6 ± 0.82 h; $p < 0.001$) and practically significant (ES = 1.07–1.11) (**Table 3**). Of the eight categories, "satisfying 7–9 sleeping hours" was the most frequent in men, whereas "dissatisfying 5–7 sleeping hours" was most frequently observed in women. For both genders, the degree of sleep satisfaction significantly increased with age ($p < 0.001$ by the Cochran–Armitage trend test) (**Table 4**).

Table 1. Average Total Sleep Duration Categorized by Gender and Age

	total sleep duration											
	men				women				overall			
age group	*n*	mean ± sd	*p* value	effect size	*n*	mean ± sd	*p* value	effect size	*n*	mean ± sd	*p* value	effect size
< 40	1158	6.0 ± 0.96			1011	6.1 ± 1.00			2169	6.1 ± 0.98		
40–49	3269	6.0 ± 0.91	<0.001 [Ψ]	0.25	2580	6.0 ± 0.93	<0.001 [Ψ]	0.16	5849	6.0 ± 0.91	<0.001 [Ψ]	0.20
50–59	3760	6.3 ± 0.94			2658	6.0 ± 0.86			6418	6.1 ± 0.93		
60 ≤	1652	6.7 ± 0.97	<0.001 [φ]		899	6.3 ± 0.97	0.0012 [φ]		2551	6.6 ± 0.98	<0.001 [φ]	
all ages	9839	6.2 ± 0.97			7148	6.0 ± 0.93			16987	6.1 ± 0.95		

[Ψ] : Kruskal–Wallis one-way analysis of variance; [φ] : Jonckheere–Terpstra trend test. Effect size describes Cohen's *f*. Two-tailed *p* value less than 0.05 was considered statistically significant.

Table 2. Associations of Coffee Intake with Total Sleep Duration and Sleep Satisfaction

coffee intake	total sleep duration				sleep satisfaction				
	n	mean ± sd	*p* value	effect size [Θ]	satisfaction	dissatisfaction	total	*p* value	effect size [τ]
rarely drink	5430	6.2 ± 1.00			3218 (59.3)	2212 (40.7)	5430 [32.0]		
1cup/day	2540	6.2 ± 0.96	<0.001 [Ψ]	0.05	1536 (60.5)	1004 (39.5)	2540 [14.9]	<0.001 [φ]	0.03
2cups/day	3647	6.1 ± 0.91	<0.001 [ξ]		2206 (60.5)	1441 (39.5)	3647 [21.5]	0.0421 [ω]	
≧ 3cups/day	5370	6.1 ± 0.93			3070 (57.2)	2300 (42.8)	5370 [31.6]		
total	16987	6.1 ± 0.95			10030 (59.0)	6957 (41.0)	16987 [100.0]		

[Ψ]: Kruskal-Wallis one-way analysis of variance; [φ]: Chi-squared test; [ξ]:Jonckheere-Terpstra trend test ; [ω]: Cochran-Armitage trend test; [Θ]: Cohen's *f*; [τ]: Cohen's *w*. () is row%, [ ] is column%, Two-tailed *p* value less than 0.05 was considered statistically significant.

Table 3. Average Sleep Duration of Subjects with or without Sleep Satisfaction, Categorized by Gender

	total sleep duration											
	men				women				overall			
sleep satisfaction	*n*	mean ± sd	*p* value	effect size	*n*	mean ± sd	*p* value	effect size	*n*	mean ± sd	*p* value	effect size
satisfaction	6185	6.6 ± 0.86	<0.001 [Ψ]	1.11	3845	6.4 ± 0.84	<0.001 [Ψ]	1.07	10030	6.5 ± 0.85	<0.001 [Ψ]	1.10
dissatisfaction	3654	5.6 ± 0.84			3303	5.6 ± 0.79			6957	5.6 ± 0.82		
total	9839	6.2 ± 0.97			7148	6.0 ± 0.93			16987	6.1 ± 0.95		

Welch's test, Effect size describe Cohen's *d*. Two-tailed *p* value less than 0.05 was considered statistically significant.

Table 4. Association between Total Sleep Duration and Sleep Satisfaction, Categorized by Gender

	sleep satisfaction								
	men			women			overall		
total sleep duration	satisfaction	dissatisfaction	total	satisfaction	dissatisfaction	total	satisfaction	dissatisfaction	total
< 5 hours	26 (9.1)	261 (90.9)	287 [2.9]	17 (7.6)	207 (92.4)	224 [3.1]	43 (8.4)	468 (91.6)	511 [3.0]
5 hours ≤ and 7 hours <	2981 (50.1)	2970 (49.9)	5951 [60.5]	2105 (43.0)	2786 (57.0)	4891 [68.4]	5086 (46.9)	5756 (53.1)	10842 [63.8]
7 hours ≤ and 9 hours <	3132 (88.2)	420 (11.8)	3552 [36.1]	1696 (84.7)	307 (15.3)	2003 [28.0]	4828 (86.9)	727 (13.1)	5555 [32.7]
9 hours ≤	46 (93.9)	3 (6.1)	49 [0.5]	27 (90.0)	3 (10.0)	30 [0.4]	73 (92.4)	6 (7.6)	79 [0.5]
total	6185 (62.9)	3654 (37.1)	9839 [100.0]	3845 (53.8)	3303 (46.2)	7148 [100.0]	10030 (59.0)	6957 (41.0)	16987 [100.0]
p value：	Chi-squared test		<0.001			<0.001			<0.001
	Cochran-Armitage trend test		<0.001			<0.001			<0.001
effect size：	Cohen's *w*		0.61			0.58			0.61

Two-tailed *p* value less than 0.05 was considered statistically significant, () is row%, [ ] is column%.

(a)

age group	men satisfaction	men dissatisfaction	men overall
<40	691 (59.7)	467 (40.3)	1158 [11.8]
40-49	1809 (55.3)	1460 (44.7)	3269 [33.2]
50-59	2387 (63.5)	1373 (36.5)	3760 [38.2]
60≤	1298 (78.6)	354 (21.4)	1652 [16.8]
all ages	6185 (62.9)	3654 (37.1)	9839 [100.0]
P value :	Chi-squared test		<0.001
	Cochran-Armitage trend test		<0.001
effect size :	Cohen's *w*		0.16

(b)

age group	women satisfaction	women dissatisfaction	women overall
<40	565 (55.9)	446 (44.1)	1011 [14.1]
40-49	1325 (51.4)	1255 (48.6)	2580 [36.1]
50-59	1363 (51.3)	1295 (48.7)	2658 [37.2]
60≤	592 (65.8)	307 (34.2)	899 [12.6]
all ages	3845 (53.8)	3303 (46.2)	7148 [100.0]
P value :	Chi-squared test		<0.001
	Cochran-Armitage trend test		<0.001
effect size :	Cohen's *w*		0.16

(c)

age group	overall satisfaction	overall dissatisfaction	overalll
<40	1256 (57.9)	913 (42.1)	2169 [12.8]
40-49	3134 (53.6)	2715 (46.4)	5849 [34.4]
50-59	3750 (58.4)	2668 (41.6)	6418 [37.8]
60≤	1890 (74.1)	661 (25.9)	2551 [15.0]
all ages	10030 (59.0)	6957 (41.0)	16987 [100.0]
P value :	Chi-squared test		<0.001
	Cochran-Armitage trend test		<0.001
effect size :	Cohen's *w*		0.16

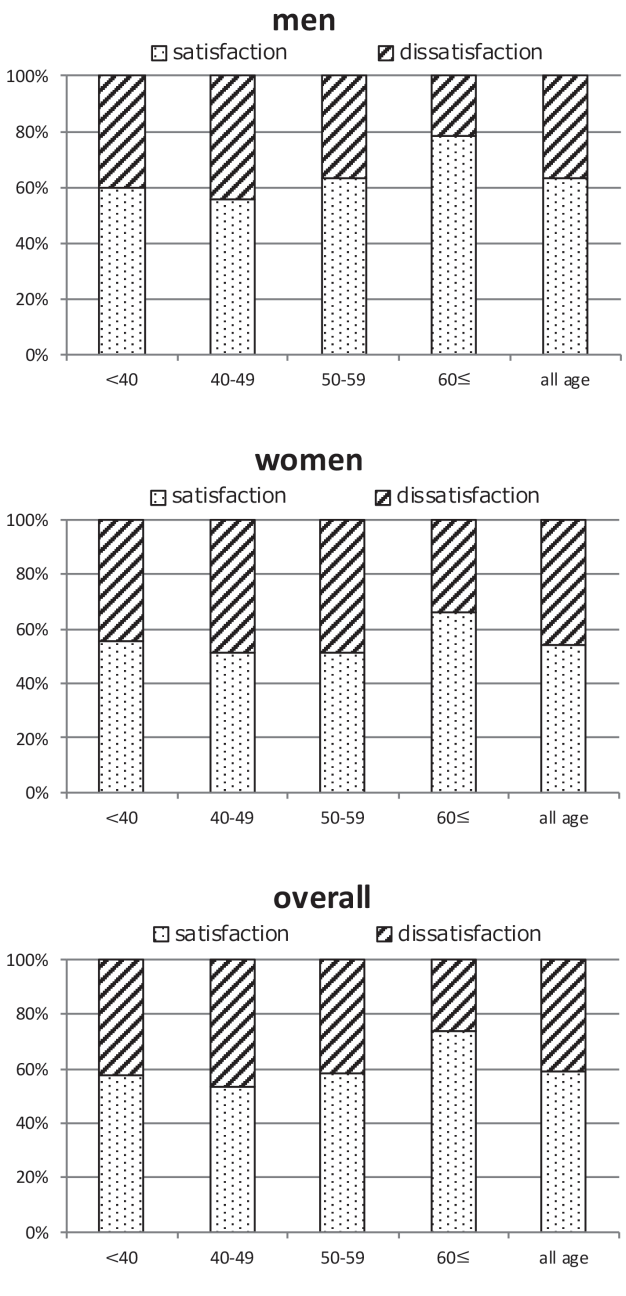


Fig.2. Sleep Satisfaction and Age Group in Men, Women, and Subjects Overall

All panels show a frequency distribution table on the left, and a histogram 100% stacked column chart on the right. Panel a shows data for men, panel b shows data for women, and panel c shows data for both genders together. Values in ( ) in the frequency distribution table are percentages for rows, and values in [ ] are percentages for columns. The effect size was estimated by Cohen's w and we used the Chi-square test to determine whether there was a statistically significant difference. Furthermore, we used the Cochran–Armitage trend test to determine whether there was a statistically significant trend.

Association of coffee intake with total sleep duration and sleep satisfaction in multivariate analyses

The results of the multivariate analyses focusing on total sleep duration and sleep satisfaction are shown in **Table 5**. Almost all background factors other than small amounts of coffee intake were associated with sleep. When focusing simply on significant factors, older age, male gender, alcohol, smoking, and fitness habits were positively associated with both total sleep duration and sleep satisfaction. In contrast, non-preferable dietary habits were negatively associated with both total sleep time and sleep satisfaction. Based on the values of standardized coefficients, the background factor with the greatest effect on total sleep duration was older age, whereas that for sleep satisfaction was having supper late at night.

With regard to coffee intake, “more than two cups per day” was negatively associated with total sleep duration,

Table 5. Summary of Estimated Total Sleep Duration and Sleep Satisfaction in Multivariate Analyses

			total sleep duration [Ψ]			sleep satisfaction [Φ]		
			Standardized Coefficient	*t*-value	*p* value	Standardized Coefficient	Odds Ratio (95% CI)	*p* value
Age			0.114	14.97	<.001*	0.128	1.14 (1.10–1.18)	<.001*
Gender	females			reference			reference	
	males		0.069	7.83	<.001*	0.139	1.15 (1.11–1.20)	<.001*
Coffee intake	non-drinker			reference			reference	
	1 cup/day		-0.005	-0.61	0.543	0.019	1.02 (0.99–1.06)	0.318
	2 cups/day		-0.019	-2.30	0.021*	0.021	1.03 (0.99–1.06)	0.274
	≧ 3 cups/day		-0.051	-6.00	<.001*	-0.035	0.97 (0.93–1.00)	0.068
BMI			-0.068	-8.89	<.001*	-0.020	0.98 (0.95–1.01)	0.246
Alcohol	rarely drinking			reference			reference	
	usually drinking		0.040	5.20	<.001*	0.087	1.09 (1.05–1.13)	<.001*
Smoking	never smoker			reference			reference	
	former smoker		0.041	4.84	<.001*	0.064	1.07 (1.03–1.11)	<.001*
	current smoker		0.055	6.30	<.001*	0.078	1.08 (1.04–1.12)	<.001*
Dietary habits	Is your speed of eating quicker than that of others?	normal		reference			reference	
		later	-0.017	-2.30	0.022*	-0.074	0.93 (0.90–0.96)	<.001*
		quicker	-0.008	-1.05	0.294	-0.042	0.96 (0.93–0.99)	0.012*
	Do you eat supper two hours before bedtime more than 3 times a week?	no		reference			reference	
		yes	-0.076	-10.13	<.001*	-0.211	0.81 (0.78–0.84)	<.001*
	Do you eat snacks after supper more than 3 times a week?	no		reference			reference	
		yes	-0.050	-6.92	<.001*	-0.118	0.89 (0.86–0.92)	<.001*
	Do you skip breakfast more than 3 times a week?	no		reference			reference	
		yes	-0.038	-5.07	<.001*	-0.080	0.92 (0.89–0.95)	<.001*
Fitness habits	Have you been doing exercise that produces light sweating for over 30 minutes a time, twice weekly, for over a year?"	no		reference			reference	
		yes	0.054	7.11	<.001*	0.166	1.18 (1.14–1.22)	<.001*
	In your daily life, do you walk or engage in an equivalent amount of physical activity more than one hour a day?	no		reference			reference	
		yes	0.013	1.70	0.09	0.096	1.10 (1.06–1.14)	<.001*
Interaction [Ω]	1 cup/day * age					-0.039	0.96 (0.93–1.00)	0.034*
	≧ 3 cups/day * BMI					0.045	1.05 (1.01–1.09)	0.021*
	≧ 3 cups/day * current smoker					-0.073	0.93 (0.89–0.97)	0.001*
	1 cup/day * Are you in a habit of doing exercise to sweat lightly for over 30 minutes a time, twice weekly, for over a year?		0.023	2.81	0.005*			
	1 cup/day * former smoker		-0.022	-2.33	0.020*			
	interactions with other variables in coffee intake				n.s.			n.s.

[Ψ]: multiple linear regression analysis; [Φ]: multiple logistic regression analysis; [Ω]: First-order interaction; n.s.: not significant; Two-tailed *p* value less than 0.05 was considered statistically significant.

with statistical significance, by the linear regression model. In contrast, there was no association between coffee intake and sleep satisfaction by the logistic regression model. Estimating the interaction between coffee intake and other variables using the linear regression model, there were statistically significant interactions in the associations of coffee intake with fitness habit and former smoker. There were also statistically significant interactions in the associations of coffee intake with age, BMI and current smoker.

Discussion

Epidemiology of sleep in Japan

According to the statistics provided by the Organisation for Economic Co-operation and Development (OECD)[24], at 456 min, the sleep duration of women in Japan was the shortest among the 26 countries surveyed. Furthermore, according to the statistics from the Survey on Time Use and Leisure Activities provided by Japan's Statistics Bureau, Ministry of Internal Affairs and Communications[38], the national average total sleep duration was 451 min, and the total sleep duration of women was significantly shorter than that of men. In both of the above surveys[24,38], total sleep duration increased with age. Differences in sleep duration between the genders were similar to those in the present research. In contrast, total sleep duration in our study was much shorter than that in the 2 surveys, probably because the percentage of middle-aged subjects (40–59 years) was higher in our study population and the Ministry of Internal Affairs and Communications survey had reported that people in 40–59 age group, considered to be those in the most productive years of working life, had a shorter sleeping time than other age groups[39].

A systematic literature review aiming to determine the right amount of sleep found that adults 26–64 years old need 7–9 h sleep each day and older adults 65 years or older need 7–8 h sleep each day[40]. Compared with its findings, the overall sleep duration in our study

population was much shorter. In addition, the rates for subjects who were dissatisfied with their sleep were very high in Japan.

Sleep durations that are too short have been reported as a risk factor for diabetes, probably due to the associated decrease in insulin sensitivity[41]. In addition, sleep deprivation has been reported to increase the risk of obesity[1–4], hypertension[10,11], cardiovascular disease [17–20], and Alzheimer's disease[22]. Furthermore, less than 6 h sleep per day has been shown to be associated with a 12% higher probability of death compared with 6–8 h sleep per day[42]. To validate these previous findings, we are planning a prospective observational study to clarify the effect of decreased sleep duration on health.

Non-preferable dietary habits should have great influence on sleep

Food intake and sleep are closely related to each other. Food intake late at night has a negative influence on sleep latency and efficiency[43]. Additionally, poor sleep-wake regularity has an undesirable effect on food intake and frequency[44,45]. Furthermore, several past studies have reported that people who have satisfactory eating habits tend to get enough sleep[46,47]. Our results from the multivariate analyses are consistent with these studies. Consequently, we believe that stopping bad dietary habits can improve quality of sleep by preventing their multiple negative effects.

Although the mechanism of the close relationship between sleep and food intake has not been adequately elucidated, two hormones may be important in this regard: ghrelin that stimulates appetite and leptin that suppresses appetite. It was reported that shorter sleep duration induces a higher serum level of ghrelin and a lower serum level of leptin[48]. It was also reported that leptin and ghrelin have positive and negative correlations, respectively, with sleep duration[49]. We think that the subtle balance between these two hormones may play an essential role in the significant association between sleep and food intake.

Effect of coffee intake upon sleep possibly minimal

Our multivariate analyses showed that coffee intake had a significant association with total sleep duration but not with sleep satisfaction. This discrepancy is very intriguing as total sleep duration and sleep satisfaction usually have a strong positive association. We suggest that the shorter sleep duration frequently observed in heavy coffee drinkers is not an undesirable effect of drinking too much coffee, but results from the intention to take advantage of its stimulant effects.

When the sizes of the effect were calculated, the Effect Size (f) of total sleep duration was 0.05, and Effect Size (ω) of sleep satisfaction was 0.03. Despite the significant association between coffee intake and sleep duration, the small values of ES suggested that the effect of coffee intake upon sleep is practically insignificant.

Limitations

The first limitation of our study is the cross-sectional design. Therefore, we were not able to accurately analyse effect sizes. The second limitation is that our study subjects were people who underwent a health check-up, so we could not evaluate the actual conditions of patients with critical disease. The third limitation is that sleep duration might have been inaccurate because it was self-reported. The fourth limitation is that we could not consider other beverages and habits that may affect sleep in these analyses.

Conclusions

Our results showed that coffee intake is significantly associated with shorter sleep duration, but not with sleep satisfaction. However, our results also showed that the effects of coffee intake upon substantial sleep are almost negligible because of their small effect sizes. Since coffee is one of the most widely consumed beverages in the world, this study will provide important, new insights in the epidemiology of sleep regarding sleep status because we have presented evidence that sleep is associated not only with coffee intake but also with typical lifestyle habits in advanced countries. These effects could vary depending on various factors such as types of coffee, place where it is drunk (home, workplace, or coffee shop), mode of drinking coffee (black, with sugar, or with cream), and time period of sleep.

Conflicts of Interest

No author reports a conflict of interest related to our manuscript.

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(Received April 18, 2018 ; Accepted May 28, 2018)

Original Article

Ningen Dock International 2019 ; 6 : 18–23

Gallbladder Adenomyomatosis is Associated with Metabolic Risk Factors, Fatty Liver, and Gallstones in Individuals Subjected to Comprehensive Health Check-ups

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Abstract

Objective: While gallbladder adenomyomatosis (GA) is generally not considered to have malignant potential, there have been several reports of gallbladder cancer arising in areas of GA. However, an association remains undefined. On the other hand, some investigators have demonstrated a clear association of GA with gallstones. Additionally, recent studies have suggested that there is a relationship between metabolic risk factors and both gallstones and fatty liver, while no studies have investigated whether similar relationships exist with GA. The purpose of our study was to determine whether GA is associated with metabolic risk factors, fatty liver, and gallstones.

Methods: A total of 3,341 subjects (1,807 males and 1,534 females) undergoing comprehensive health check-ups with no history of cholecystectomy, hypertension, diabetes, dyslipidemia, and/or hyperuricemia underwent abdominal ultrasonography. Using univariate and multivariate analyses, parameters associated with GA as well as associations between GA, fatty liver, and gallstones were examined by sex.

Results: Subjects with GA (58 males and 28 females) exhibited significantly worse metabolic parameters compared to those without GA (1,749 males and 1,506 females) in univariate analyses. After adjustments for other factors, age, waist circumference, and smoking habit remained associated with GA in males, while in females, associations with age, body mass index, hemoglobin A1c levels, uric acid, and smoking habit became significant. Furthermore, fatty liver and gallstones remained associated with GA after adjusting for other risk factors.

Conclusion: Our analyses suggest that fatty liver, gallstones, and GA are strongly associated with metabolic risk factors, while GA, is closely related with smoking habit, fatty liver, and gallstones.

Keywords gallbladder adenomyomatosis, metabolic risk factors, fatty liver, gallstones

Gallbladder adenomyomatosis (GA) is an abnormality of the gallbladder characterized by overgrowth of the muscle layer and the mucosa. Branched tubular projections of the mucosa into the muscle layer and Rokitansky-Aschoff sinuses produce a histological appearance simulating gland and cyst formation. GA has been referred to as adenomyoma[1], cholecystitis glandularis proliferans[2], and intramural diverticulosis[3]. The term GA was introduced by Jutras *et al.*[4] to differentiate it from inflammatory conditions such as cholecystitis, because GA lacks inflammatory features but exhibits features of hyperplasia.

While GA is generally not considered to be a premalignant condition, there have been several reports[5–8] of gallbladder cancer in patients with GA. However, this association has not been a consistent finding in published series[5–11].

In contrast, some investigators[10–12] have shown that there is a close association of GA with gallstones. Additionally, recent studies[13–16] have suggested that gallstones and fatty liver are strongly associated with metabolic risk factors. However, no study has yet evaluated a potential association between GA and metabolic risk factors. The purpose of our study was to determine whether GA is associated with metabolic risk factors, fatty liver, and gallstones in individuals undergoing a comprehensive health check-up.

Methods

This study was approved by the Ethics Committee of the Yokohama City Minato Red Cross Hospital. The need to obtain informed consent from the subjects was

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waived by the Ethics Committee.

Subjects

Between June 2011 and September 2016, a total of 4,598 individuals undergoing a comprehensive health check-up who had no history of cholecystectomy were subjected to abdominal ultrasonography. After excluding subjects with a history of hypertension, diabetes, dyslipidemia, and/or hyperuricemia, 3,341 subjects (1,807 males and 1,534 females, aged 17 to 92 years) were enrolled in a cross-sectional observational study. The initial data of the subjects who underwent multiple check-ups during the above period were used.

Measurements

The comprehensive health check-up included recording the subject's medical history, prescribed medications, and lifestyle habits such as alcohol use, exercise, and smoking habit; determination of anthropometric variables; a physical examination; and laboratory tests. Weight and height were measured with the subject dressed in light clothing and without shoes. The body mass index (BMI) was defined as weight in kilograms divided by height squared in meters (kg/m^2). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using an automated sphygmomanometer in the sitting position. The measurement of waist circumference (WC) was performed at the level of the umbilicus after normal expiration while standing. Venous blood samples were collected after an overnight fast of at least 12 hours. The blood samples were used for analysis of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), and uric acid (UA). Obesity was defined as BMI ≥ $25 kg/m^2$. Male and female subjects were diagnosed with abdominal obesity (AO) if they had a WC ≥ 85 cm or ≥ 90 cm, respectively. High SBP was defined as SBP ≥ 130 mmHg, high DBP as DBP ≥ 85 mmHg, high LDL-C as LDL-C ≥ 120 mg/dL, low HDL-C as HDL-C ≤ 39 mg/dL, high TG as TG ≥ 150 mg/dL, high FPG as FPG ≥ 100 mg/dL, high HbA1c as HbA1c ≥ 5.6 %, and high UA as UA ≥ 7.1 mg/dL.

Assessment of Gallbladder adenomyomatosis, fatty liver, and gallstones

Experienced technicians performed abdominal ultrasonography for each subject using a 3.5 MHz transducer to assess evidence of GA, fatty liver, and gallstones non-invasively and safely. The ultrasonographic images were reviewed by a gastroenterologist (Ito M) blinded to the individual's data. GA was defined on the basis of the generally accepted findings: a thickened gallbladder wall, a small cystic area, and a comet-like echo. Fatty liver was diagnosed based on the presence of four acknowledged findings: liver brightness, vascular blurring, hepatorenal contrast, and deep attenuation. Gallstones were defined as echogenic foci that cast an acoustic shadow and sought gravitational dependency (movable structures), or a sludge that was echogenic in appearance but did not cast an acoustic shadow or was gravitationally dependent because of its viscosity.

Statistical analysis

All analyses were performed using SPSS statistics, version 24 (IBM Japan, Ltd., Tokyo, Japan). A two-tailed p value < 0.05 was considered significant. To compare differences between subjects with and without GA by sex, the unpaired t-test was used for quantitative variables (expressed as the mean ± standard deviation) when normally distributed, Mann-Whitney's U test was used for non-normally distributed values, and the chi-squared test was used for categorical variables (presented as absolute numbers and percentages). Furthermore, stepwise logistic regression analyses were performed to determine which parameters were independently associated with GA. To avoid the influence of other relevant factors, further stepwise logistic regression analyses were conducted to analyze the relationship between GA and fatty liver or gallstones.

Results

The background characteristics of the subjects with and without GA are presented in **Table 1**. Of the 3,341 subjects 86 (2.6%) had GA. Of the 1,807 males and 1,534 females, 58 (3.2%) and 28 (1.8%) had GA, respectively. Overall, fatty liver was observed in 893 (26.7%) subjects: 672 (37.2%) males, and 221 (14.4%) females. Gallstones were detected in 199 (6.0%) subjects: 126 (7.0%) in males and 73 (4.8%) in females. All three diseases evaluated in this study were more prevalent in males than females. Subjects with GA had worse metabolic parameters compared with those without GA. Age, BMI, WC, SBP, and DBP were significantly higher in subjects with GA than in those without GA, both in males and in females. Furthermore, comparing female subjects with and without GA, statistically significant differences were observed for LDL-C, HDL-C, TG, and UA levels. Obesity, AO, high SBP, high DBP, fatty liver, gallstones, and smoking habit were significantly more prevalent in subjects with GA than in those without GA in both males and females. Females with GA had significantly higher frequencies of high LDL-C, low HDL-C, high TG, high HbA1c, and high UA than females without GA.

The results of the multivariate logistic regression analysis of metabolic risk factors for GA are shown in **Table 2**. Age, WC, and smoking habit were independently associated with GA in males, while in females GA was associated with age, BMI, HbA1c, UA, and smoking habit.

Table 3 shows the association between GA and fatty

Table 1. Background Characteristics of Subjects with and without GA

	Males			Females		
	Without GA (n = 1749)	With GA (n = 58)	p value	Without GA (n = 1506)	With GA (n = 28)	p value
Age (years)	51.4 ± 12.1	55.3 ± 9.5	0.003 **	51.0 ± 12.1	59.3 ± 12.2	< 0.001 **
BMI (kg/m^2)	23.5 ± 3.0	24.6 ± 3.3	0.008 **	21.2 ± 3.1	24.2 ± 3.4	< 0.001 **
WC (cm)	83.9 ± 8.2	88.1 ± 9.8	< 0.001 **	77.7 ± 8.6	85.3 ± 9.7	< 0.001 **
SBP (mmHg)	121.0 ± 15.1	126.1 ± 14.5	0.013 **	112.0 ± 15.3	122.2 ± 15.0	< 0.001 **
DBP (mmHg)	76.2 ± 10.3	80.2 ± 10.1	0.004 **	70.2 ± 9.8	75.4 ± 10.2	0.006 **
LDL-C (mg/dL)	125.7 ± 31.1	129.8 ± 31.7	0.321	120.6 ± 32.0	137.2 ± 28.6	0.007 **
HDL-C (mg/dL)	56.1 ± 13.3	53.9 ± 13.1	0.222	68.2 ± 15.0	62.3 ± 16.1	0.041 *
TG (mg/dL)	124.6 ± 99.7	140.8 ± 87.4	0.221	84.3 ± 58.0	118.3 ± 57.4	0.002 **
FPG (mg/dL)	97.8 ± 12.8	100.0 ± 13.2	0.194	92.0 ± 10.9	99.8 ± 30.2	0.185
HbA1c (%)	5.6 ± 0.5	5.7 ± 0.4	0.456	5.6 ± 0.4	6.1 ± 1.4	0.078
UA (mg/dL)	6.2 ± 1.2	6.2 ± 1.2	0.972	4.6 ± 1.0	5.6 ± 1.4	0.001 **
Obesity (%)	487 (27.8%)	23 (39.7%)	0.049 *	171 (11.4%)	9 (32.1%)	0.001 **
AO (%)	708 (40.5%)	35 (60.3%)	0.002 **	127 (8.4%)	6 (21.4%)	0.015 *
High SBP (%)	432 (24.7%)	24 (41.4%)	0.004 **	194 (12.9%)	12 (42.9%)	< 0.001 **
High DBP (%)	328 (18.8%)	20 (34.5%)	0.003 **	127 (8.4%)	7 (25.0%)	0.002 *
High LDL-C (%)	992 (56.7%)	36 (62.1%)	0.418	730 (48.5%)	20 (71.4%)	0.016 *
Low HDL-C (%)	127 (7.3%)	7 (12.1%)	0.169	16 (1.1%)	2 (7.1%)	0.003 *
High TG (%)	419 (24.0%)	17 (29.3%)	0.348	112 (7.4%)	9 (32.1%)	< 0.001 **
High FPG (%)	657 (37.6%)	27 (46.6%)	0.165	267 (17.7%)	7 (25.0%)	0.320
High HbA1c (%)	981 (56.1%)	39 (67.2%)	0.092	857 (56.9%)	22 (78.6%)	0.022 *
High UA (%)	396 (22.6%)	14 (24.1%)	0.781	14 (0.9%)	4 (14.3%)	< 0.001**
Fatty liver (%)	634 (36.2%)	38 (55.5%)	< 0.001 **	204 (13.5%)	17 (60.7%)	< 0.001**
Gallstones (%)	107 (6.1%)	19 (32.8%)	< 0.001 **	66 (4.4%)	7 (25.0%)	< 0.001 **
Without exercise habit (%)	868 (49.6%)	37 (63.8%)	0.034 *	843 (56.0%)	14 (50.0%)	0.528
With smoking habit (%)	447 (25.6%)	25 (43.1%)	0.003 **	133 (8.8%)	6 (21.4%)	0.021 *
With alcohol habit (%)	625 (35.7%)	20 (34.5%)	0.845	194 (12.9%)	3 (10.7%)	0.734

GA: gallbladder adenomyomatosis, BMI: body mass index, WC: waist circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglycerides, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c, UA: uric acid, AO: abdominal obesity. Age is shown as mean ± standard deviation (SD). * : p <0.05, ** : p < 0.01

Table 2. Variables Associated with GA in Multivariate Logistic Regression Analysis

	RC	SE	odds ratio	95% CI	p value
Males					
Age	0.036	0.012	1.036	1.013–1.060	0.002 **
WC	0.055	0.015	1.057	1.025–1.089	< 0.001 **
With smoking habit	0.873	0.281	2.395	1.380–4.156	0.002 **
Females					
Age	0.053	0.017	1.054	1.019–1.090	0.002 **
BMI	0.167	0.052	1.182	1.067–1.309	0.001 **
HbA1c	0.622	0.210	1.863	1.234–2.812	0.003 **
UA	0.494	0.163	1.639	1.191–2.255	0.002 **
With smoking habit	1.085	0.523	2.961	1.063–8.250	0.038 *

GA: gallbladder adenomyomatosis, RC: regression coefficient, SE: standard error, CI: confidence interval, WC: waist circumference , BMI: body mass index, HbA1c: hemoglobin A1c, UA: uric acid. * : p <0.05, **: p < 0.01

liver or gallstones adjusted for other risk factors. Not only gallstones but also fatty liver remained associated with GA in both males and females after adjustments for other risk factors.

Discussion

The present study showed that subjects with GA exhibited significantly worse metabolic parameters and metabolic disorders were more prevalent than in subjects without GA according to univariate analyses.

Table 3 Relationship between GA and Fatty Liver or Gallstones Adjusted for Other Risk Factors

	RC	SE	odds ratio	95% CI	*p* value
Males					
Fatty liver	1.215	0.284	3.370	1.933–5.877	< 0.001 **
Gallstones	1.965	0.303	7.137	3.941–12.922	< 0.001 **
Females					
Fatty liver	1.644	0.445	5.176	2.164–12.380	< 0.001 **
Gallstones	1.409	0.533	4.092	1.441–11.624	0.008 **

GA: gallbladder adenomyomatosis, RC: regression coefficient, SE: standard error, CI: confidence interval
* : p <0.05, ** : p < 0.01

Moreover, age, WC, and smoking habit remained significantly associated with GA in males, while age, BMI, HbA1c, UA, and smoking habit became significantly associated for female subjects after adjustment for other risk factors. Furthermore, fatty liver and gallstones remained associated with GA both in males and females after adjusting for other risk factors. These results suggest that GA is closely related with metabolic abnormalities such as obesity, smoking habit, fatty liver, and gallstones.

The present study is the only one so far to have focused on the relationship between GA and smoking habit. Our finding that GA is strongly associated with smoking habit is unexpected and stimulates interest and discussion.

Although King *et al.*[2] previously proposed that adenomyomatosis is a consequence of chronic inflammation, and named this condition cholecystitis glandularis proliferans, the pathogenesis of GA is still unknown. Inflammation is not always present, particularly when the lesion is localized at the fundus[17]. Regarding pressure-related colonic diverticula, Rokitansky-Aschoff sinuses are most likely to be found where the muscle layer is weakest (i.e. at the site of a penetrating blood vessel). It has been postulated that increased intraluminal pressure in the gallbladder resulting from obstruction by calculi, a congenital septum, an acquired kink in the cystic duct, or gallbladder dyskinesia promote cystic dilatation of the Rokitansky-Aschoff sinuses[4]. In addition, several investigators have observed an association between adenomyomatosis and an anomalous pancreaticobiliary ductal union. In one study, half of the patients with adenomyomatosis had an anomalous pancreaticobiliary ductal union[18], and in another study, one third of patients with an anomalous pancreaticobiliary ductal union had adenomyomatosis[19]. Thus, the pathogenic mechanism of GA remains unclear.

Our study suggests that tobacco may be a risk factor for GA. Tobacco might accelerate and intensify mechanical and/or functional dysfunction of the gallbladder. Additionally, some studies[20–22] have demonstrated that the other two diseases addressed in this study, fatty liver and gallstones, were also associated with smoking, while other studies[13,23–25] have reported that no relationship existed between them and smoking. Our previous study[20] indicated that not only metabolic disorders, such as AO, but also smoking habit were independently associated with non-alcoholic fatty liver disease (NAFLD). Thus, the influence of tobacco on these three diseases remains to be elucidated.

As described above, fatty liver and gallstones were significantly associated with GA in both males and females even after adjusting for other risk factors. These results suggest that GA, fatty liver, and gallstones might share common risk factors and pathogenic mechanisms. Koller *et al.*[13] reported that NAFLD was an independent risk factor for cholelithiasis and might represent a pathogenetic link between metabolic syndrome and cholelithiasis. Furthermore, Asai *et al.*[16] verified that activation of the hypoxia inducible factor 1α subunit pathway in the steatotic liver concentrates biliary lipids via the suppression of water secretion from hepatocytes and increases the formation of cholesterol gallstones. In contrast, studies on the pathogenesis of GA have not produced significant results. This may be due to the fact that GA is considered a benign disease and does not attract much interest from investigators. Longitudinal studies are therefore needed to clarify the factors or entities that exist before GA emerges and to identify the potential risk factors for GA.

Our results indicating that the frequencies of the three diseases, GA, fatty liver, and gallstones, were higher in males than in females, are consistent with previous studies[15,26]. The acknowledged finding[27,28] that the prevalence of metabolic syndrome is higher in males than in females might be a causative factor for these diseases, because all three of them are associated with metabolic risk factors. Intriguingly, the present study indicated that the number of metabolic risk factors related to GA was higher in females than in males not only in univariate analyses but also in multivariate analyses. Liu *et al.*[29] reported that NAFLD was more associated with gallstones in females than males in a longitudinal cohort study in a Chinese urban popula-

tion. While the influence of sex on gallbladder diseases remains unclear, the present study showed that GA is strongly associated with metabolic parameters in both males and females.

Our study has several limitations. First, the diagnosis of GA was based on ultrasound imaging. The absence of a gold standard for the diagnosis of GA contributes to lack of accuracy. Secondly, the fact that our study population included individuals subjected to abdominal ultrasound as part of a comprehensive health check-up may have led to a selection bias, and may not be indicative of the general population. Finally, because the study design was cross-sectional, we can only suggest that there is an association between GA and metabolic abnormalities, fatty liver, and gallstones. Longitudinal studies are now needed.

Conclusions

Our analysis suggests that not only fatty liver and gallstones but also GA is strongly associated with metabolic risk factors, and in particular, GA is closely associated with smoking habits, fatty liver, and gallstones.

An abstract of this paper was presented at the 58th Congress of the Japan Society of Ningen Dock held in August 2017.

Conflicts of Interest

The authors state that they have no conflicts of interest to disclose.

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(Received April 20, 2018 ; Accepted June 4, 2018)

Original Article

Ningen Dock International 2019 ; 6 : 24–28

Salt Intake is Closely Associated with Body Weight in Patients with Lifestyle-related Diseases

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Abstract

Objective: The aim of this study was to assess daily salt intake and associated factors in patients with lifestyle-related diseases.

Methods: The participants were 132 men and 123 women who regularly visited our center for treatment of lifestyle-related diseases. Salt intake was estimated using 24-h urine collections without adjustment for non-urinary losses.

Results: Men and women consumed an average of 13.1 and 10.2 g/day of salt per day ($p<0.001$), respectively. Calculation of salt intake per kg of body weight (BW) revealed that there was no difference between men and women. The correlation coefficient between salt intake and BW was higher than that between it and body mass index, body height, and standard BW. Multiple regression analyses revealed that salt intake was most closely associated with BW, followed by frequency of drinking and age. When adjusted for frequency of drinking and age, the salt intake of participants with BWs in the highest quartile was approximately 1.5 times greater than those with BWs in the lowest quartile.

Conclusion: These results suggest that restricting daily salt intake is more difficult for individuals who require a higher amount of total energy than those with lower total energy requirements.

Keywords sodium, salt intake, body weight, lifestyle-related diseases

The average daily salt intake among Japanese has gradually decreased, from 11.5 g in 2005 to 10.0 g (11.0 g in men, 9.2 g in women) in 2015[1].

However, Japanese salt intake remains high compared with countries in Europe, North and South America, and Africa[2]. In 2015, Japan's Ministry of Health, Labour and Welfare recommended a daily salt intake of <8 g in men and <7 g in women[3], while the World Health Organization reported that salt intake in adults should be <5 g[4]. For people with hypertension, the Japanese Society of Hypertension[5] recommends an intake <6 g and the American Heart Association[6] recommends an intake of <3.8 g. These recommended intakes are independent of individual total energy requirements.

The Japan Diabetes Society places emphasis on a balance of nutrients and recommends that 50–60% of total energy requirements come from carbohydrates, no more than 20% from protein, and the remainder from lipids[7]. Naturally, individuals with higher energy requirements consume more food, and therefore more salt, assuming the foods they consume are seasoned to the same degree as those eaten by individuals with lower energy requirements.

In this study, we estimated daily salt intake using 24-h urine collections in patients with lifestyle-related diseases, and examined factors associated with salt intake.

Methods

Participants were patients who regularly visited our center for treatment of lifestyle-related diseases including diabetes, hypertension, and/or dyslipidemia, who participated in 24-h urine collection to assess daily salt intake. This study was approved by the ethics committee of Ageo Central General Hospital (No. 183, date of approval: March 26, 2013). Verbal consent for the analytical use of anonymized date was obtained from all participants. Salt intake was estimated without adjustment for non-urinary losses from 24-h urine collections, which were conducted from January to March in 2014. The

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amount of daily salt intake (g/day) was calculated using the following equation: urinary sodium concentration (mEq/L) × urine volume (L/day) × 58.5/1000. The expected creatinine excretion (mg/day) estimated from sex, age, body height (BH), and body weight (BW) was calculated according to the following equation: for men, BW (kg) × 15.12 + BH (cm) × 7.39 − age × 12.63 − 79.90; for women, BW (kg) × 8.58 + BH (cm) × 5.09 − age × 4.72 − 74.95[5]. If the percentage of the actual amount of urinary creatinine of the expected amount was <70% or >130%, collections were excluded from analyses. Other exclusion criteria were proteinuria ≥ 0.5 g/day and creatinine≥1.3 mg/dL in men and≥ 1.2mg/dL in women. The final population whose urine collections were analyzed consisted of 132 men and 123 women. Participants with hypertension had been advised to reduce their daily salt intake to <6 g by a dietitian or a doctor. However, those without hypertension did not receive this advice.

Statistical analyses were performed using Dr SPSS II for Windows (IBM Japan, Ltd., Tokyo, Japan). Student's *t*-test and one-way analysis of variance were used to compare the means of two and three groups, respectively. Pearson's method was used to calculate correlation coefficients. Analysis of covariance was conducted to adjust for differences in some variables. Multiple regression analyses were performed to determine independent associated factors. Differences with a *p*-value <0.05 were considered significant.

Results

Participants' average ages were approximately the same for men (68.3 years old) and women (68.9 years old). Compared with women, men had a significantly higher BH and BW, but no significant difference in body mass index (BMI) was found (**Table 1**). Regarding lifestyle habits, fewer men than women had never smoked, while more men than women were current smokers. The frequencies of drinking and exercise were higher in men than women. All participants were taking at least one medication, to treat one or more of the following: diabetes (59.6%), hypertension (69.8%), and dyslipidemia (61.2%).

Daily salt intake was significantly different between men (13.1 g) and women (10.2 g) (**Table 1**). However, no difference was observed when salt intake was calculated per 1 kg of BW or standard BW. **Table 2** shows unadjusted and age-adjusted correlation coefficients between salt intake and variables related to body size. The correlation coefficient between salt intake and BW was higher than that between it and BMI, BH, and standard BW. We conducted multiple regression analyses selecting BW and BMI from body size parameters as explanatory variables (**Table 3**). Salt intake was most closely associated with BW, followed by frequency of drinking and age. No association was found between salt intake and consumption of antihypertensive drugs. When participants were stratified according to BW quartiles, salt intake was greatest in the highest quartile (**Table 4**). When adjusted for frequency of drinking and age,

Table 1. Participants' Clinical Chracteristics and Daily Salt Intake

	Men	Women	*p*
n	132	123	
Age	68.3 (7.7)	68.9 (9.4)	
BH (cm)	166.1 (6.0)	152.2 (6.1)	***
BW (kg)	67.2 (10.7)	55.2 (9.5)	***
BMI (kg/m^2)	24.4 (3.4)	23.8 (3.6)	
Drinking (times/week)	3.0 (3.2)	0.4 (1.5)	***
Exercise (times/week)	3.8 (2.7)	3.1 (2.8)	*
Smoking			
never (%)	12.1	87.8	***
past (%)	74.2	8.1	***
present (%)	13.6	4.1	**
Medication			
diabetes (%)	57.6	61.8	
hypertension (%)	69.7	69.9	
dyslipidemia (%)	56.1	66.7	
Salt intake /day			
g	13.1 (5.2)	10.2 (3.5)	***
g/kg BW	0.194 (0.068)	0.187 (0.056)	
g/kg SBW	0.215 (0.082)	0.201 (0.066)	

Variables are given as means ± SD. *: $p < 0.05$, ** < 0.01, *** < 0.001
BW: body weight, BH: body height, SBW: standard body weight
BMI: body mass index

Table 2. Correlation Coefficients between Daily Sodium Intake and Participants' Body Size Variables

	Age	BW (kg)	BH (cm)	SBW (kg)	BMI (kg/m^2)
Unadjusted	-0.279	0.502	0.371	0.373	0.347
Adjusted for age	–	0.453	0.331	0.333	0.302

BW: body weight, BH: body height, SBW: standard body weight, BMI: body mass index

Table 3. Multiple Regression Analysis for Salt Intake

	Salt (g/day)	
	T value	*p*
Sex (female to male)	-0.228	0.820
Age (years)	-2.055	0.041
BMI (kg/m^2)	-0.368	0.714
BW (kg)	3.434	0.001
Smoking (present to never and past)	-1.421	0.157
Drinking (times/week)	2.209	0.028
Exercise (times/week)	-1.031	0.304
Antihypertensive drug (with to without)	-1.417	0.158

BMI: body mass index, BW: body weight

Table 4. Participants' Characteristics and Salt Intake Stratified According to Quartiles of Body Weight

BW (kg)	< 54	54–59	60–66	=/> 67	*p*
n	61	59	69	66	
Age	70.7 (8.8)	70.5 (8.1)	69.2 (7.3)	64.2 (8.5)	***
BH (cm)	151.3 (7.3)	156.3 (6.8)	162.3 (7.3)	166.7 (7.0)	***
BW (kg)	47.6 (4.4)	56.5 (1.7)	63.0 (2.2)	77.0 (8.3)	***
BMI (kg/m^2)	20.9 (2.5)	23.3 (2.0)	24.1 (2.4)	27.8 (2.9)	***
Drinking (times/week)	0.7 (1.9)	1.2 (2.6)	2.3 (3.1)	2.7 (3.1)	***
Exercise (times/week)	3.5 (2.9)	3.1 (2.6)	3.9 (2.9)	3.3 (2.7)	
Smoking					
never (%)	77.0	62.7	34.8	24.2	***
past (%)	21.3	33.9	52.2	59.1	***
present (%)	1.6	3.4	13.0	16.7	**
Medication					
diabetes (%)	55.7	54.2	56.5	71.2	
hypertension (%)	62.3	72.9	75.4	68.2	
dyslipidemia (%)	52.5	66.1	62.3	63.6	
Salt intake/day (unadjusted)					
g	9.4 (2.8)	11.2 (3.7)	11.0 (3.6)	15.0 (6.0)	***
g/kg BW	0.196 (0.054)	0.199 (0.066)	0.175 (0.056)	0.195 (0.071)	
Salt intake/day (adjusted for age and the frequency of drinking)$					
g	9.8 (0.5)	11.5 (0.5)	11.0 (0.5)	14.4 (0.5)	***
g/kg BW	0.202 (0.008)	0.203 (0.008)	0.174 (0.007)	0.186 (0.008)	*

Variables are given as means ± SD except for $ where variables are given as means ± SE. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$
BW: body weight, BH: body height, BMI: body mass index

salt intake in the highest quartile was approximately 1.5 times greater than in the lowest quartile.

Discussion

Our results indicate that salt intake is very closely associated with BW. Among our study participants, salt intake in men was significantly greater than in women, though no differences were observed when intake was adjusted for BW. Partial correlation coefficients and multiple regression analyses revealed a close association between BW and salt intake. Salt intake among participants with BW in the highest quartile was approximately 1.5 times greater than that in the lowest quartile. Individuals with higher BWs are generally thought to

eat more food to meet their total energy requirements, resulting in a higher salt intake compared with those with lower BWs.

However, the daily salt intakes recommended by academic societies are independent of individual total energy requirements. For example, the Japanese Society of Hypertension recommends a salt intake of < 6 g daily for people with hypertension[5] and the recommendation of the Japan Diabetes Society is < 6 g for diabetic patients with stage 3–5 nephropathy[8]. Therefore, it is likely that achieving the targets for daily salt intake is more difficult for individuals who require more energy than for those who require less.

Clinical trials have shown the difficulty in reducing sodium intake levels[9–11]. In the Trial of Hypertension Prevention (TOHP)-II, 24-h urinary sodium excretion in the intervention group was 2.5 g at 6 months and 3.1 g at 36 months, despite the target sodium intake being 1.8 g[11]. The extended post-trial surveillance of the TOHP trial revealed that only 1.4% of participants consumed <1.5 g sodium per day and 10% consumed <2.3 g/day[12]. This study suggested overall health benefits from reducing sodium intake to the 1.5–2.3 g/day range. However, whether a sodium intake of <1.5 g/day is safe remains unclear because data are sparse for this level of intake. Some studies have shown a J-shaped association between cardiovascular events and 24-h urinary sodium excretion, which was estimated from fasting morning specimens instead of 24-h urine collections[13,14]. Pooled analysis of data from four studies by Mente *et al.* showed that in both normotensive and hypertensive populations, sodium excretion of <3 g/day was associated with increased risk of cardiovascular events and death compared with sodium excretion of 4–6 g/day[14]. An association between high cardiovascular mortality and low sodium intake has also been reported in both type 1 and type 2 diabetes, where sodium intake was estimated from 24-h urine collections[15,16]. Although the mechanisms of this J-shaped association are unknown, an interesting hypothesis is that low sodium intake activates the renin system[17], resulting in increased cardiovascular events[14,18].

In this study, we estimated sodium intake from 24-h urine collections without adjusting for sodium excretion from routes other than urine. Holbrook *et al.* reported that 86% of sodium consumed orally was excreted into the urine[19] and Mickelsen *et al.* that 93.4% of sodium was recovered in urine during cool weather[20]. Considering excretion rates into urine, salt intake in our participants was markedly greater than the average Japanese salt intake obtained from the 2015 National Health and Nutrition Survey[1], which was based on household-level records[21]. This discrepancy may partly be explained by differences in measurement methods. Dietary questionnaires reportedly underestimate sodium intake, compared with 24-h urinary collections[22]. The amount of salt intake in our participants was close to amounts reported by Asakura *et al.*, which were estimated from 24-h urine collections[23].

One result that deserves consideration is that no difference in salt intake was observed between participants consuming and those not consuming antihypertensive medications. Long, long-term adherence to low sodium intake reportedly tends to be poor[10,11]. Therefore, it would be worth repeating urinary sodium measurements to encourage salt restriction. Another result that deserves consideration is that salt intake in our participants was negatively associated with age. This result is different from that of the 2015 National Health and Nutrition Survey[1], in which salt intake was greatest in the sixth decade of life. This difference may be explained by the fact that our participants had lifestyle-related diseases and were more careful about their health at higher ages.

In summary, we have shown a close association between sodium intake and BW. It is likely that achieving the level of salt intake recommended by academic societies is more difficult for individuals with higher energy requirements than for those with lower energy requirements. In the Dietary Approaches to Stop Hypertension Sodium trial, which investigated the effects of three different amounts of sodium intake on blood pressure, sodium levels were defined as those included in energy intakes of 2,100 kcal, and sodium intakes were proportionate to total energy requirements of individual participants[9]. It would be important to elucidate whether daily salt intakes appropriate for individuals are independent of their total energy requirements.

Conflict of Interest

The authors have no conflict of interest to declare.

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(Received June 6, 2018 ; Accepted July 2, 2018)

Original Article

Ningen Dock International 2019 ; 6 : 29–36

Hypercholesterolemia is Suggested to be an Independent Risk Factor of Incident Hearing Loss in Japanese Men and Women Undergoing Health Screening

Eiji Oda

Abstract

Background and Aims: Hypercholesterolemia has been suggested to be a risk factor of hearing loss. However, most of the epidemiological studies showing this were cross-sectional and controversial. The aim of this longitudinal study was to find independent risk factors of incident hearing loss.

Methods: This was an 8-year follow-up study to find risk factors of high-frequency hearing loss (HFHL) and low-frequency hearing loss (LFHL) in 2,628 and 2,775 participants, respectively. The threshold levels of HFHL and LFHL were defined at the 40 dB (4,000 Hz) and the 30 dB (1,000 Hz) for a better ear, respectively. Age and sex adjusted hazard ratios (HRs) of incident hearing loss were calculated for candidate risk factors. Then, stepwise Cox regressions were performed. Similar calculations were made excluding subjects older than 60 years.

Results: HFHL and LFHL developed in 18.4% of men and 6.5% of women and 8.7% of men and 9.5% of women, respectively. The incidence of hearing loss was markedly higher in participants older than 60 years: 53.4% in men and 20.6% in women and 28.4% in men and 26.9% in women, respectively, for HFHL and LFHL. Smoking and daily alcohol drinking were independent risk factors of HFHL while hypercholesterolemia was an independent risk factor of LFHL for all participants. Hypercholesterolemia and smoking were independent risk factors of HFHL while hypercholesterolemia and anemia were independent risk factors of LFHL after excluding subjects older than 60 years.

Conclusions: Our results suggested that hypercholesterolemia was an independent risk factor of both HFHL and LFHL after excluding subjects older than 60 years. Individuals with hypercholesterolemia should be recommended to undergo audiometry and avoid risk factors of hearing loss.

Keywords incident hearing loss, hypercholesterolemia, anemia, smoking

The World Health Organization has reported that 466 million people worldwide have disabling hearing loss (HL), and 1.1 billion young people are at risk of HL due to exposure to noise in recreational settings[1]. HL may result from genetic causes, complications at birth, chronic ear or systemic infections, certain drugs, exposure to excessive noise and ageing. Unaddressed HL poses an annual global cost of 750 billion international dollars, so interventions to prevent, identify and address HL are cost-effective and can bring great benefit to individuals with HL from early identification[1]. So, it is important to explore risk factors of HL. However, there have been conflicting reports of associations between HL and possible risk factors. Most of the studies concerning risk factors of HL have been cross-sectional[2–18].

Among longitudinal studies, one reported that hypertension, diabetes and obesity were not associated with incident HL while smoking and hypercholesterolemia had small but significant associations with incident HL in men[19]. Another study reported a significant association between hypercholesterolemia and incident sudden sensorineural HL (SSHL)[20] and yet another reported a significant association between hypertension and incident HL in women[21]. There has also been a study that reported a significant association between incident HL and diabetes and a borderline association between incident HL and prediabetes[22]. Furthermore, there have been experimental studies whose results suggested that hypercholesterolemia may be a risk factor of HL[23–27].

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The aim of the present study was to find risk factors of incident HL in a health screening population.

The study was approved by the ethics committee of Tachikawa General Hospital and was performed in accordance with the ethical standards of the Declaration of Helsinki in 1964 and its later amendments.

Subjects and Methods

Subjects

Among 3,866 individuals who visited our Medical Check-up Center for general health screening between April 2008 and March 2009 and gave written informed consent to use their data for epidemiological studies, 3,716 individuals (37% women) aged 24–82 years completed pure tone audiometry. They were all required to fill out a questionnaire recommended by the Japanese Ministry of Health, Labor and Welfare, which includes questions on histories of coronary heart disease and stroke, smoking and drinking status, physical activity, antihypertensive, antidiabetic, and antihyperlipidemic medications. After excluding individuals with a history of coronary heart disease or stroke, 3,541 remained. Further excluding individuals with high-frequency HL (HFHL) and low-frequency HL (LFHL) at baseline left 3,204 and 3,383 participants as potential subjects for the present incident HFHL and LFHL study, respectively. Owing to drop-outs during the 8-year follow-up period, 2,628 and 2,775 participants were actually involved in the present incident HFHL and LFHL study, respectively.

Measurements

Pure tone audiometry was performed by trained technicians using an AA-46 system (RION Co. Ltd., Tokyo, Japan). LFHL and HFHL were diagnosed using a threshold level of 30 dB at 1,000 Hz and one of 40 dB at 4,000 Hz, respectively. After an overnight fast, blood samples were obtained to measure blood levels of routine health screening tests including fasting plasma glucose (FPG), triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, HbA1c, uric acid, hemoglobin, high-sensitivity C-reactive protein (hs-CRP) and creatinine. Measurements were performed at BML Nagaoka (Nagaoka, Japan) using routine laboratory methods except for hs-CRP, which was measured at BML General Laboratory (Tokyo, Japan) by nephelometry using N-latex CRP-2 (Siemens Healthcare K.K., Tokyo, Japan). The measurement limit of hs-CRP was 0.02 mg/L and levels of hs-CRP less than the measurement limit were considered to be 0.01 mg/L. LDL cholesterol was measured using a direct surfactant method with Choletest-LDL (SEKISUI MEDICAL CO., LTD, Tokyo, Japan). HbA1c was measured by latex aggregation immunoassay using Determiner HbA1c (Kyowa Medex Co., Ltd., Tokyo, Japan) and expressed in NGSP%. Average systolic blood pressure (SBP) and diastolic blood pressure (DBP) were calculated from two automatic measurements using a MPV-3301 instrument (NIHON KOHDEN CORPORATION, Tokyo, Japan) in the sitting position after a 5 min rest. Body height and weight were automatically measured using a TBF-210 device (TANITA corporation, Tokyo, Japan) wearing light clothes provided by our center, subtracting the weight of the clothing from the measured body weight. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Respiratory function tests, including percent vital capacity (%VC) and forced expiratory volume in 1 second divided by forced vital capacity (FEV1/FVC), were performed by trained clinical technicians using the Autospirometer System 7 (MINATO MEDICAL SCIENCE CO., LTD., Osaka, Japan). The spirometry methods complied with the guidelines for spirometry examinations issued by the Japanese Respiratory Society. Slow vital capacity was measured several times. %VC was calculated as the maximal slow vital capacity divided by predicted vital capacity. Predicted vital capacity (PVC) was calculated using the equations recommended by the Japanese Respiratory Society in 2001: PVC = $0.045 \times \text{height (cm)}^{-0.023} \times \text{age (years)}^{-2.258}$ in men and $0.032 \times \text{height (cm)}^{-0.018} \times \text{age (years)}^{-1.178}$ in women. Estimated glomerular filtration rate (eGFR) was calculated from serum creatinine using the gender-specific equations for Japanese recommended by the Japanese Society of Nephrology[28].

Definition of candidate risk factors

Candidate risk factors were defined as below.

Obesity-25: BMI ≥ 25 kg/m^2.

Obesity-30: BMI ≥ 30 kg/m^2.

Hypertension: SBP ≥ 140 mmHg or DBP ≤ 90 mmHg or use of antihypertensive drugs.

Diabetes: FPG ≥ 7.0 mmol/L or HbA1c ≥ 6.5% or use of antidiabetic drugs.

Prediabetes: FPG ≥ 5.6 mmol/L or HbA1c ≥ 5.7% excluding diabetes.

Hypercholesterolemia: total cholesterol ≥ 6.2 mmol/L or use of antihyperlipidemic drugs.

Hyper-LDL cholesterolemia-1: LDL cholesterol ≥ 3.6 mmol/L or use of antihyperlipidemic drugs.

Hyper-LDL cholesterolemia-2: LDL cholesterol ≥ 4.1 mmol/L or use of antihyperlipidemic drugs.

Hypertriglyceridemia: triglycerides ≥ 1.7 mmol/L.

Hypo-HDL cholesterolemia: HDL cholesterol < 1.0 mmol/L in men and < 1.3 mmol/L in women.

Hyperuricemia: ≥ 400 μmol/L in men and ≥ 360 μmol/L in women.

Anemia: hemoglobin < 130 g/L in men and < 120 g/L in women.

Low-grade inflammation: hs-CRP ≥ 1.00 mg/L.

Renal dysfunction (RD): eGFR < 60 mL/min/1.73m^2.

Restrictive lung disease (RLD): %VC < 80%.
Obstructive lung disease (OLD): FEV1/FVC < 70%.

Obesity-25 includes Obesity-30. Hypo-HDL cholesterolemia was defined differently by sex according to the international definition[29] although the definition is the same for both sexes in Japan. Detailed information about antihyperlipidemic drugs was not available but most of the antihyperlipidemic drugs were considered to have been prescribed for hypercholesterolemia. Therefore, antihyperlipidemic drugs were all considered to be antihypercholesterolemic drugs in this study. This assumption is not exactly scientific but was a practical way of dealing with the limited information.

Detailed quantitative information was not obtained regarding alcohol consumption in this study.

Statistical analysis

Baseline variables were compared between participants who developed HFHL or LFHL during the follow-up period and their normal counterparts. Physical activity was defined as walking for one hour or longer per day or exercising for 30 minutes or longer twice or more per week. Means were compared by *t*-tests and percentages were compared by *chi*-squared tests.

Calculated incidences of HFHL and LFHL were stratified by age groups (decades) and sex.

Hazard ratios (HRs) of LFHL and HFHL were calculated adjusting for sex and age using Cox regression models, in which years were used as the unit of the survival variable, the first diagnosis with hearing loss by annual audiometry was ascertained as the outcome and subjects without the outcome were censored at their last annual health screening visit. Then, stepwise Cox regressions were performed using $p < 0.05$ as the inclusion criterion and $p \geq 0.1$ as the exclusion criterion adopting age, male sex, current smoking, daily alcohol drinking, physical activity and all the candidate risk factors defined above as the initial covariates. The above calculations of HRs were repeated excluding participants older than 60 years.

Statistical analyses were performed using Dr SPSS-2 (IBM Japan, Tokyo, Japan). *p* values of lower than 0.05 were considered significant.

Results

Baseline data stratified by development of HL are presented in **Table 1**. Male sex, age, obesity-25, hypertension, diabetes, prediabetes, hypercholesterolemia, low-grade inflammation, RD, OLD, current smoking, daily alcohol drinking and physical activity were significantly associated with incident HFHL while age, hypertension, diabetes, prediabetes, hypercholesterolemia, hyper-LDL cholesterolemia-1, hyper-LDL cholesterolemia-2, low-grade inflammation, RD, OLD and physical activity were significantly associated with incident LFHL.

Incidences of HFHL and LFHL are shown in **Table 2** stratified by age groups (decade) and sex. HFHL developed in 303 men (18.4%) and 64 women (6.5%) while LFHL developed in 153 men (8.7%) and 32 women (9.4%) during the 8 years of follow-up. The mean follow-up duration was 5.7 years in the HFHL study and 5.9 years in the LFHL study. The incidences of HFHL and LFHL were markedly higher in the age groups with subjects older than 60 years.

The HRs (95% confidence intervals (CIs)) of HFHL and LFHL for candidate risk factors are shown in **Table 3**. There were no significant associations between incident HFHL and obesity-25, hypertension, diabetes, prediabetes, hypercholesterolemia, low-grade inflammation, RD, OLD or physical activity after adjustment for sex and age. Only current smoking and daily alcohol drinking were significantly associated with incident HFHL after adjustment for sex and age. There were no significant associations between incident LFHL and hypertension, diabetes, prediabetes, hyper-LDL cholesterolemia-1, hyper-LDL cholesterolemia-2, low-grade inflammation, RD, OLD or physical activity after adjustment for sex and age. Only hypercholesterolemia was significantly associated with incident LFHL after adjustment for sex and age. Age, male sex, current smoking and daily alcohol drinking were significantly associated with incident HFHL in the final step of the stepwise regressions while age and hypercholesterolemia were significantly associated with incident LFHL in the final step of the stepwise regressions.

The HRs (95% CIs) of HFHL and LFHL for candidate risk factors calculated excluding participants older than 60 years are shown in **Table 4**. Among the 2,259 (63.2% men) participants, 218 (9.7%) subjects developed HFHL and among the 2,380 (63.7% men) participants, 138 (5.8%) subjects developed LFHL. There were significant associations between incident HFHL and hypercholesterolemia, hyper-LDL cholesterolemia-1 and current smoking after adjustment for sex and age. Only hypercholesterolemia was significantly associated with incident LFHL after adjustment for sex and age. There were significant associations between incident HFHL and age, male sex, hypercholesterolemia and current smoking in the final step of the stepwise regressions while age, hypercholesterolemia and anemia were significantly associated with incident LFHL in the final step of the stepwise regressions.

Discussion

In the present 8-year follow-up study, current smoking and daily alcohol drinking were independently associated with incident HFHL while hypercholesterolemia was independently associated with incident LFHL, in a health screening population. After excluding indi-

Table 1. Baseline Variables Stratified by Incident Hearing Loss

	high-frequency audiometry			low-frequency audiometry		
	normal	impaired	*p*	normal	impaired	*p*
n	2261	367		2527	248	
men (%)	59.6	82.6	<0.001	63.4	61.3	0.512
age (years)	50.0 (8.7)	59.4 (8.4)	<0.001	50.6 (8.9)	59.7 (9.0)	<0.001
obesity-25 [a] (%)	19.4	25.9	0.004	19.9	24.2	0.109
obesity-30 [b] (%)	2.1	2.5	0.646	2.1	2.0	0.932
hypertension (%)	21.5	35.1	<0.001	23.0	33.1	<0.001
diabetes (%)	3.6	8.7	<0.001	4.1	6.9	0.040
prediabetes (%)	22.6	32.2	<0.001	23.2	33.9	<0.001
hypercholesterolemia [c] (%)	19.5	24.5	0.026	19.4	31.0	<0.001
hyper-LDLC-1 [d] (%)	30.9	35.1	0.102	31.0	39.5	0.006
hyper-LDLC-2 [e] (%)	16.5	20.2	0.079	16.4	24.2	0.002
hypertriglyceridemia (%)	16.5	19.9	0.108	16.9	17.3	0.872
hypo-HDLC [f] (%)	8.0	7.6	0.805	7.9	8.1	0.934
hyperuricemia (%)	20.5	19.1	0.535	20.3	16.5	0.157
low-grade inflammation [g] (%)	12.1	16.9	0.010	12.4	18.1	0.010
anemia (%)	8.4	6.3	0.172	7.8	10.5	0.131
renal dysfunction (%)	4.6	9.0	<0.001	4.8	11.7	<0.001
restrictive lung disease (%)	4.1	4.9	0.484	4.0	5.6	0.201
obstructive lung disease (%)	2.1	5.7	<0.001	2.5	4.8	0.030
current smoking (%)	23.1	29.4	0.008	24.7	19.8	0.084
daily alcohol drinking (%)	36.0	51.0	<0.001	37.8	41.1	0.308
physical activity [h] (%)	34.9	41.1	0.020	34.9	41.9	0.028
antihypertensives (%)	12.5	24.0	<0.001	13.5	22.6	<0.001
antidiabetics (%)	1.8	5.2	<0.001	2.2	4.0	0.073
antihyperlipidemic (%)	7.4	12.8	<0.001	7.6	16.9	<0.001
body mass index (kg/m^2)	22.5 (3.0)	23.2 (3.0)	<0.001	22.6 (3.0)	23.1 (3.0)	0.025
SBP [i] (mmHg)	117.4 (17.6)	123.0 (18.3)	<0.001	118.1 (17.8)	121.2 (18.1)	0.010
DBP [j] (mmHg)	74.2 (11.0)	77.6 (11.1)	<0.001	74.6 (11.1)	76.2 (11.2)	0.041
FPG [k] (mmol/L)	5.15 (0.73)	5.44 (1.09)	<0.001	5.18 (0.78)	5.36 (0.91)	0.001
hemoglobin A1c (%)	5.4 (0.5)	5.6 (0.6)	<0.001	5.4 (0.5)	5.6 (0.5)	<0.001
total cholesterol (mmol/L)	5.29 (0.82)	5.27 (0.79)	0.612	5.29 (0.80)	5.38 (0.84)	0.088
LDL cholesterol (mmol/L)	3.15 (0.75)	3.11 (0.74)	0.411	3.14 (0.75)	3.20 (0.74)	0.215
HDL cholesterol (mmol/L)	1.60 (0.40)	1.53 (0.40)	0.004	1.59 (0.40)	1.56 (0.39)	0.286
triglycerides (mmol/L)	1.19 (0.76)	1.31 (0.88)	0.006	1.20 (0.78)	1.23 (0.64)	0.602
uric acid (μ mol/L)	327 (83)	339 (83)	0.012	327 (83)	321 (77)	0.108
hs-CRP [l] (mg/L)	0.55 (1.00)	0.97 (3.61)	<0.001	0.59 (1.45)	0.70 (1.17)	0.239
hemoglobin (g/L)	141 (15)	144 (12)	<0.001	142 (15)	141 (14)	0.276
eGFR [m] (mL/min/1.73m^2)	79.1 (12.8)	75.9 (13.0)	<0.001	79.1 (12.8)	74.8 (14.3)	<0.001
percent vital capacity (%)	96.5 (11.3)	96.9 (11.5)	0.508	96.8 (11.3)	97.0 (12.3)	0.712
FEV1/FVC [n] (%)	81.6 (5.9)	78.8 (6.8)	<0.001	81.3 (6.1)	79.9 (6.6)	0.001

mean (SD) or %, [a] BMI ≥ 25 kg/m^2, [b] BMI ≥ 30 kg/m^2, [c] total cholesterol ≥ 6.2 mmol/L or use of antihyperlipidemic drugs, [d] LDL cholesterol ≥ 3.6 mmol/L or use of antihyperlipidemic drugs, [e] LDL cholesterol ≥ 4.1mmol/L or use of antihyperlipidemic drugs, [f] hypo-HDL cholesterolemia, [g] hs-CRP ≥ 1 mg/dL, [h] defined as walking for one hour or longer per day or exercising for 30 minutes or longer twice or more per week, [i] systolic blood pressure, [j] diastolic blood pressure, [k] fasting plasma glucose, [l] high-sensitivity CRP, [m] estimated glomerular filtration rate, [n] forced expiratory volume in 1 second divided by forced vital capacity, *t*-tests for means and chi-squared tests for percentages

viduals older than 60 years, hypercholesterolemia and current smoking were independently associated with incident HFHL and hypercholesterolemia and anemia were independently associated with incident LFHL in the stepwise regressions. However, anemia might be questionable as an independent risk factor because sex was excluded from the covariates by the stepwise regression in the LFHL study. The present study suggests that old age may confound associations between risk factors and HL because it is a very strong risk factor of HL as shown in **Table 2**.

Associations between HL and risk factors are controversial and most of the epidemiological studies have been cross-sectional[2–18]. Hyperlipidemia was thought to be a risk factor of HL in animal experiments [23–27].

Among longitudinal studies, one evaluated associations between hypertension, diabetes, hypercholesterolemia, smoking and BMI and incident HL in 26,917

Table 2. Incidence of Hearing Loss Stratified by Sex and Age Groups (Decades)

	high-frequency hearing loss				low-frequency hearing loss			
	men		women		men		women	
age (years)	n	incidence (%)	n	incidence (%)	n	incidence (%)	n	incidence (%)
–30	9	0.0	4	0.0	9	0.0	4	0.0
31–40	221	2.3	134	1.5	232	0.9	135	3.0
41–50	551	8.0	311	1.9	579	3.6	321	3.4
51–60	646	20.9	383	6.8	695	8.8	405	9.6
subtotal	1427	12.9	832	4.1	1515	5.6	865	6.2
61–70	185	48.6	120	20.8	197	26.9	127	25.2
71–80	34	79.4	25	20.0	38	36.8	28	35.7
81–	4	50.0	1	0.0	4	25.0	1	0.0
subtotal	223	53.4	146	20.6	239	28.4	156	26.9
total	1650	18.4	978	6.5	1754	8.7	1021	9.4

Table 3. Hazard Ratios of Hearing Loss

	high-frequency hearing loss		low-frequency hearing loss	
	hazard ratio (95% CI [h])	*p*	hazard ratio (95% CI [h])	*p*
adjusted for sex and age				
obesity-25 [a]	1.169 (0.925–1.479)	0.191	1.198 (0.895–1.605)	0.225
obesity-30 [b]	1.661 (0.855–3.226)	0.134	1.210 (0.498–2.941)	0.674
hypertension	0.921 (0.737–1.152)	0.472	0.978 (0.742–1.288)	0.872
diabetes	1.194 (0.828–1.722)	0.342	1.121 (0.681–1.847)	0.653
prediabetes	1.078 (0.864–1.344)	0.507	1.089 (0.834–1.422)	0.533
hypercholesterolemia [c]	1.159 (0.911–1.476)	0.230	1.334 (1.013–1.785)	0.041
hyper-LDLC-1 [d]	1.105 (0.891–1.371)	0.364	1.190 (0.919–1.540)	0.186
hyper-LDLC-2 [e]	1.166 (0.901–1.510)	0.242	1.205 (0.895–1.623)	0.218
hypertriglyceridemia	1.101 (0.849–1.428)	0.468	1.140 (0.816–1.594)	0.443
hypo-HDL cholesterolemia	1.010 (0.687–1.485)	0.960	1.098 (0.695–1.735)	0.688
hyperuricemia	0.872 (0.669–1.136)	0.309	0.957 (0.678–1.349)	0.801
low-grade inflammation [f]	1.204 (0.915–1.583)	0.185	1.322 (0.955–1.831)	0.092
anemia	0.957 (0.624–1.470)	0.842	1.226 (0.811–1.853)	0.333
renal dysfunction	0.928 (0.644–1.337)	0.688	1.190 (0.799–1.771)	0.392
restrictive lung disease	0.847 (0.525–1.365)	0.494	1.033 (0.600–1.780)	0.906
obstructive lung disease	0.921 (0.587–1.444)	0.719	1.071 (0.594–1.931)	0.820
current smoking	1.461 (1.153–1.850)	0.002	1.083 (0.778–1.507)	0.637
daily alcohol drinking	1.345 (1.082–1.671)	0.008	1.277 (0.965–1.691)	0.087
physical activity [g]	1.090 (0.884–1.344)	0.419	1.112 (0.863–1.434)	0.411
final step of stepwise regressions [i]				
age (years)	1.115 (1.103–1.128)	<0.001	1.105 (1.091–1.120)	<0.001
male sex	2.739 (2.055–3.651)	<0.001		
current smoking	1.402 (1.104–1.781)	0.006		
daily alcohol drinking	1.289 (1.035–1.606)	0.024	1.244 (0.962–1.608)	0.096
hypercholesterolemia [c]			1.384 (1.054–1.818)	0.019
low-grade inflammation [f]			1.330 (0.961–1.840)	0.086

[a] BMI ≥ 25 kg/m^2, [b] BMI ≥ 30 kg/m^2, [c] total cholesterol ≥ 6.2 mmol/L or use of antihyperlipidemic drugs, [d] LDL cholesterol ≥ 3.6 mmol/L or use of antihyperlipidemic drugs, [e] LDL cholesterol ≥ 4.1 mmol/L or use of antihyperlipidemic drugs, [f] hs-CRP ≥ 1 mg/dL, [g] defined as walking for one hour or longer per day or exercising for 30 minutes or longer twice or more per week, [h] confidence interval, [i] using age, sex, current smoking, daily alcohol drinking, physical activity, obesity-25, obesity-30, hypertension, diabetes, prediabetes, hypercholesterolemia, hyper-LDLC-1, hyper-LDLC-2, hypertriglyceridemia, hypo-HDL cholesterolemia, hyperuricemia, low-grade inflammation, anemia, chronic kidney disease, restrictive lung disease, obstructive lung disease as the initial covariates, Blank cells in the table indicate that *p* for the covariate in the left column was higher than 0.1.

men and reported that hypertension, diabetes and obesity were not associated with incident HL while smoking and hypercholesterolemia had a small but significant association with incident HL[19]. A longitudinal case-control study in Taiwan suggested a significant association between hypercholesterolemia and incident SSHL[20]. The present study observed a significant association between hypercholesterolemia and both HFHL

Table 4. Hazard Ratios of Hearing Loss Excluding Subjects Older than 60 Years

	high-frequency hearing loss		low-frequency hearing loss	
	hazard ratio (95% CI [h])	*p*	hazard ratio (95% CI [h])	*p*
adjusted for sex and age				
obesity-25 [a]	1.233 (0.916–1.659)	0.168	1.198 (0.895–1.605)	0.225
obesity-30 [b]	1.749 (0.823–3.718)	0.146	1.210 (0.498–2.941)	0.674
hypertension	0.921 (0.681–1.246)	0.594	0.978 (0.742–1.288)	0.872
diabetes	1.162 (0.685–1.973)	0.577	1.121 (0.681–1.847)	0.653
prediabetes	1.222 (0.914–1.634)	0.175	1.089 (0.834–1.422)	0.533
hypercholesterolemia [c]	1.457 (1.071–1.980)	0.016	1.334 (1.013–1.758)	0.041
hyper-LDLC-1 [d]	1.373 (1.044–1.805)	0.023	1.190 (0.919–1.540)	0.186
hyper-LDLC-2 [e]	1.361 (0.982–1.884)	0.064	1.205 (0.895–1.623)	0.218
hypertriglyceridemia	1.162 (0.844–1.601)	0.357	1.140 (0.816–1.594)	0.443
hypo-HDL cholesterolemia	1.173 (0.740–1.859)	0.496	1.098 (0.695–1.735)	0.688
hyperuricemia	0.908 (0.659–1.253)	0.558	0.957 (0.678–1.349)	0.801
low-grade inflammation [f]	1.119 (0.752–1.664)	0.580	1.322 (0.955–1.831)	0.092
anemia	0.916 (0.466–1.802)	0.800	1.226 (0.811–1.853)	0.333
renal dysfunction	1.122 (0.640–1.969)	0.688	1.190 (0.799–1.771)	0.392
restrictive lung disease	1.111 (0.570–2.167)	0.757	1.033 (0.600–1.780)	0.906
obstructive lung disease	1.431 (0.706–2.904)	0.320	1.071 (0.594–1.931)	0.820
current smoking	1.692 (1.278–2.241)	<0.001	1.083 (0.778–1.507)	0.637
daily alcohol drinking	1.081 (0.817–1.430)	0.587	1.277 (0.965–1.691)	0.087
physical activity [g]	1.036 (0.782–1.373)	0.806	1.112 (0.863–1.434)	0.411
final step of stepwise regressions [i]				
age (years)	1.154 (1.125–1.184)	<0.001	1.124 (1.090–1.159)	<0.001
male sex	3.015 (2.054–4.426)	<0.001		
hypercholesterolemia c	1.524 (1.120–2.075)	0.007	1.644 (1.146–2.361)	0.007
anemia			1.775 (1.036–3.042)	0.037
current smoking	1.739 (1.312–2.305)	<0.001		

[a] BMI ≥ 25 kg/m^2, [b] BMI ≥ 30 kg/m^2, [c] total cholesterol ≥ 6.2 mmol/L or use of antihyperlipidemic drugs, [d] LDL cholesterol ≥ 3.6 mmol/L or use of antihyperlipidemic drugs, [e] LDL cholesterol ≥ 4.1 mmol/L or use of antihyperlipidemic drugs, [f] hs-CRP ≥ 1 mg/dL, [g] defined as walking for one hour or longer per day or exercising for 30 minutes or longer twice or more per week, [h] confidence interval, [i] using age, sex, current smoking, daily alcohol drinking, physical activity, obesity-25, obesity-30, hypertension, diabetes, prediabetes, hypercholesterolemia, hyper-LDLC-1, hyper-LDLC-2, hypertriglyceridemia, hypo-HDL cholesterolemia, hyperuricemia, low-grade inflammation, anemia, chronic kidney disease, restrictive lung disease, obstructive lung disease as the initial covariates, Blank cells in the table indicate that *p* for the covariate in the left column was higher than 0.1.

and LFHL after excluding individuals older than 60 years. Smoking was also significantly associated with HFHL after adjustment for confounders in the present study. Another study reported a small but significant association between hypertension and incident HL[21] and yet another reported a borderline association of incident HL with prediabetes and a significant association with diabetes as compared with normoglycemia[22]. However, there was no significant association between incident HL and hypertension, prediabetes or diabetes in the present study even after excluding participants older than 60 years.

Cholesterol stabilizes the cell membrane and modulates lipid and protein translocation across it. In the cochlea, the lipid composition, fluidity, and stiffness of the outer hair cell lateral wall membrane have been shown to be important to its function[25,26]. Ultrastructural analysis of cochleas from hypercholesterolemic chinchillas has revealed alterations in both the stria vascularis and outer hair cells and these alterations suggested that chronic hypercholesterolemia metabolically stresses inner ear tissue[27]. Hypercholesterolemia may also decrease cochlear vascularity and contribute to HL.

The present study suggested that hypercholesterolemia, but not hyper-LDL cholesterolemia, was a significant risk factor of HL after excluding subjects older than 60 years. Thus, cholesterol in lipoproteins other than LDL may contribute to the development of HL. Our results suggested that hypercholesterolemia was a significant risk factor of LFHL, not HFHL, in all subjects. The incidence of HFHL in men was 2–3 times higher than that in women while the incidence of LFHL was almost equal in men and women, as shown in **Table 2**. The influence of hypercholesterolemia on HL may be stronger in women than men.

Limitations

The participants of the present study were not recruited from a general population and detailed information regarding history of otological diseases, noise exposure, demographic backgrounds and medications

was not available. Therefore, residual confounders might have influenced the present results. The number of participants may have been too small to detect significant associations between incident HL and hyper-LDL cholesterolemia. Also, exclusion of antihypercholesterolemic drug users may have left an insufficient number of participants for detecting significant associations between incident HL and hypercholesterolemia (data not shown).

Conclusions

The present 8-year follow-up study suggested that hypercholesterolemia was significantly associated with both incident HFHL and incident LFHL after adjusting for confounders in a health screening population excluding subjects older than 60 years. Individuals with hypercholesterolemia should be recommended to undergo audiometry for early detection of hearing loss and to avoid risk factors of hearing loss such as exposure to noise.

Conflict of Interest

The author has no conflict of interest.

Acknowledgments

The author received no financial support. He wishes to thank all subjects who participated in the study as well as the paramedical staff at their center who assisted with it.

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(Received June 6, 2018 ; Accepted September 11, 2018)

Original Article

Ningen Dock International 2019 ; 6 : 37–43

Mammographic Breast Density: Comparison of Fully Automated Quantitative Assessment (Volpara™) with Visual Qualitative Classification in a Japanese Population and Investigation of Factors Influencing Disagreement

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Abstract

Objective: In Japan, to achieve sensitivity in mammography, a current approach is to use ultrasonography together with mammography for dense breasts. Although more objective and reproducible determination of dense breasts is necessary, few studies have shown correlations between radiologists' assessments and fully automated volumetric measurements. This study was undertaken to assess the level of agreement between the two evaluation methods and to identify factors associated with disagreement between them.

Methods: Between April and June 2017, 1,456 women were included in the study. Agreement in breast density assessments between two radiologists and automated software was evaluated using kappa (κ) values. We also evaluated factors that could have contributed to any differences using univariate and multivariate analysis.

Results: Inter-observer agreement was good (κ=0.701). Breast density assessments made by radiologists versus software exhibited fair agreement (κ=0.224). Age, menopausal stage, difference in bilateral breast density, fibroglandular tissue volume, breast tissue volume and volumetric breast density (VBD) were significantly different between the two methods in univariate analysis. In multivariate analysis, VBD was an independent correlative factor for greater possibility of significant disagreement (Odds ratio for agreement = 0.755; 95% CI, 0.730–0.782).

Conclusions: Automated volumetric breast density measurements showed fair agreement with radiologists' visual assessments. Low VBD was a contributing factor in disagreement. Although the automated method may have value, further research is necessary in Japan before it is put into widespread use.

Keywords mammography, Volpara, automated volumetric breast density measurement, dense breast

Mammographic breast density is important. First, there is the risk of dense breasts masking tumors and they reduce the sensitivity of mammography in detecting breast cancer. Second, increased breast density is significant risk factor for developing breast cancer[1–6].

In Japan, to achieve sensitivity in mammography, a current approach is to use ultrasonography together with mammographic examination for dense breasts. A randomized trial (Japan Strategic Anti-cancer Randomized Trial: J-START) was conducted to evaluate the effectiveness of breast ultrasound in breast cancer screening for 40-year-old Japanese women. The breast cancer detection rate rose approximately 1.5 times by using ultrasound in combination with mammography[7]. Based on this result, research and discussion continues regarding whether ultrasound will be performed in the future as a population-based screening tool for dense breasts, which may easily mask tumors. Furthermore, classification of dense breasts is expected to be more important in the future.

The most commonly used clinical classification of mammographic density is the qualitative Breast Imaging-Reporting and Date System (BI-RADS)[8]. In Japan, mammographic density is classified into four levels in the Japan Central Organization on Quality Assurance of Breast Cancer Screening, based on BI-RADS[9]. The BI-RADS and Japanese classifications are shown **Table**

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1 and **2**. However, it has been suggested that there is variable intra- and inter-observer agreement in subjective assessment of mammographic density, causing a lack of reliability in the assigned BI-RADS density categories[10,11]. To improve objectivity and reproducibility, quantitative breast density measurements have been developed. In Japan, automated breast density measurements are beginning to be introduced, and are expected to provide a method of objectively determining dense breasts. However, few studies have investigated whether breast densities assessed by radiologists correlate with those obtained from fully automated volumetric measurements. Notably, there has been no study on this issue in Japanese women.

Therefore, the purpose of this study was to assess agreement on mammographic density between the fully automated volumetric method and radiologists' determinations based on the classifications in Japanese women, and to identify factors influencing disagreement between the two methods.

Methods

Study population

Because of the retrospective nature of the study, a waiver of informed written consent and ethical approval was approved by the Institutional Review Board of our institution. We perform approximately 13,000 mammography inspections per year in our facility. A total of 2,298 women underwent screening mammograms between April 2017 and June 2017. Exams on women with breast cancer and history of breast surgery, and those for which insufficient data was available were excluded. The final number of women included in this study was 1,456 (mean age: 51.9 ± 8.9 years). Of the 1,456 exams, 855 had medial lateral oblique (MLO) views only, while 601 had both MLO and cranio-caudal (CC) views (Senographe pristina: GE Healthcare).

Radiologist assessments

The 1,456 exams were retrospectively classified according to the Japanese breast density categories (**Table 2**) by two radiologists with more than ten years of experience in breast imaging. The coincidence rate between the two radiologists was calculated and exams in which evaluations by the two radiologists were consistent were extracted. We reclassified exams with a score of one or two as "Fatty", and those with a score of three or four as "Dense".

Volumetric breast density analysis by Volpara

A total of 1,465 exams were assessed using fully automated volumetric breast density measurement software: Volpara™ (version 1.5.1.1, Matakina Technology, Wellington, New Zealand). Volpara produces a fibroglandularity content map of the breast that allows estimation of breast density measurement. Volumetric breast density (VBD) refers to the percentage of breast density, computed by dividing fibroglandular tissue volume by breast volume. Breast density was a continuous value, ranging from 0% to approximately 40%. VDG (Volpara density grade) categories were assigned automatically, according to the respective VBD values. Values for exams that had both MLO and CC views were obtained by averaging the two results. A VBD value of 0 – 4.7% corresponds to VDG a, 4.8 – 7.9% to VDG b, 8.0 – 15.0% to VDG c, and >15.1% to VDG d[12]. The thresholds of the VDG categories were determined by an American expert group of radiologists by recording the average VBD for the assigned BI-RADS category in 500 mammography examinations. These were further revised based on BI-RADS 5th edition in 2013[13].

VDG was categorized in similar manner to that for the radiologist assessments: VDG categories a and b were included in a Fatty group and VDG categories c and d were included in a Dense group. We further

Table 1. BI-RADS Classification of Breast Density (4th and 5th)

BI-RADS 4th	Bi-RADS 5th
1. the breast is almost entirely fat.	a. The breasts are almost entirely fatty.
2. scattered fibroglandular densities. (25 – 50%)	b. There are scattered areas of fibroglandular density.
3. heterogeneously dense breast tissue. (51 – 75%)	c. The breasts are heterogeneously dense, which may obscure small masses.
4. extremely dense.(> 75% glandular)	d. The breasts are extremely dense, which lowers the sensitivity of mammography.

Table 2. Japanese Classification of Breast Density

1. The breast is almost all fat. The detection of lesions is easy.
2. There are scattered fibroglandular densities. The detection of lesions is easy. (fat in breast approximately 70 – 90%)
3. Fat is mixed in with breast substance and it presents heterogeneous density, which could obscure detection of masses. (fat in breast approximately 40 – 50%)
4. There is little fat mixed in with breast substance, and the rate of detection of lesions is low. (fat in breast approximately 10 – 20%)

classified the exams of subjects into an agreement and a disagreement group based on the evaluations according to radiologists and VDG. Namely, if the evaluation by radiologists was consistent with that according to VDG, the subjects were included in the agreement group. Conversely, if the evaluation by radiologists was inconsistent with that according to VDG, these subjects were included in the disagreement group. We also examined factors that could have contributed to differences between the two groups. The factors were patient age, menopause stage, difference in bilateral breast density, mammographic final assessment (Japanese category 1 to 5), fibroglandular tissue volume, breast volume, and VBD. We defined difference in bilateral breast density as a situation in which the grade difference in VDG category between the right and left breast was ≥1. In addition, we defined mammographic final assessment categories 1 and 2 as negative findings and categories 3, 4, and 5 as positive findings.

Statistical analysis

Inter-observer agreement was assessed using kappa (κ) values for the four density classes. Kappa (κ) values were also used to assess agreement between observers and Volpara for the two additional density classes (Fatty/Dense).

According to convention, strengths of agreement were expressed as κ values: a value of 0.2 or less indicated agreement was poor, 0.21 – 0.40 it was fair, 0.41 – 0.60 it was moderate, 0.61 – 0.80 it was good, and 0.81 – 1.00 it was very good[14]. Factors that may have contributed to differences between the agreement and disagreement groups were evaluated by univariate analysis and multivariate analysis using logistic regression. For the univariate analysis, we performed *t*-tests for continuous variables such as age, and the chi-square test for non-continuous variables, such as presence of menopause, mammographic final assessment, and difference in bilateral breast density, as assessed by VDG. We used the Wilcoxon rank-sum test for fibroglandular tissue volume, breast volume, and VBD. We also performed multivariate analysis to estimate the odds ratio and 95% confidence intervals (CIs) for the factors with significant differences in univariate analysis. The dependent variable had agreement group as 1. All calculations were performed using the SPSS software (IBM: SPSS statistics, version 24).

Results

Baseline variables are given in **Table 3**. The mean (SD) age at mammography examination was 51.9 (8.9) years. There were 703 (48%) post-menopausal women. The mammographic final assessment was determined as "negative" in 1,424 cases (98%) and "positive" in 32 cases (2%). Regarding Volpara density measurements per examination, the median fibroglandular tissue volume was 51.1 cm^3 (range 3.3 – 275.9), median breast volume was 327.2 cm^3 (range 4.2 – 1,568.2), and median VBD was 15.2% (range 2.2 – 42.0). The frequencies of recorded densities according to the two radiologists and VDG are given in **Table 4**. Cases where both radiologists assessed breasts as dense accounted for 40% of the total; while the rate for assessment by Volpara was approximately 85%. An example is shown in **Fig. 1**. Inter-observer agreement is given in **Table 5**. Agreement was good (observed agreement 79%, κ =0.701). We extracted 1,152 cases in which the evaluations of the two radiologists were consistent, from the 1,456 cases. There was fair agreement between visual assessment and VDG (observed agreement 56%, κ=0.224; **Table 6**). There were very few cases where

Table 3. Baseline Variables

Age mean, years (SD)	51.9 (8.9)
Menopause stage Postmenopausal (%)	703 (48)
Fibroglandular tissue volume median, cm^3 (range)	51.1 (3.3 – 275.9)
Breast tissue volume median, cm^3 (range)	327.2 (4.2 – 1568.2)
VBD median, % (range)	15.2 (2.2 – 42.0)

Table 4. Frequency of Breast Density Categories as Assessed by Radiologist 1, Radiologist 2, and Volpara

Visual category	1	2	3	4	total
Radiologist 1 (%)	44 (3)	807 (56)	587 (40)	18 (1)	1456
Radiologist 2 (%)	63 (4)	792 (55)	599 (41)	2 (0)	1456
	a	b	c	d	total
Volpara (%)	20 (1)	201 (14)	520 (36)	715 (49)	1456

Cases in which both radiologists assessed breasts as dense accounted for 40% of the total. On the other hand, the rate for Volpara was about 85%.

radiologists made an assessment of Dense when the assessment by Volpara was Fatty; however, their visual assessment of approximately half of the cases assessed as Dense by Volpara was Fatty. To investigate this mismatch, we extracted 960 cases that had been evaluated by Volpara as Dense (c, d) and visually compared the

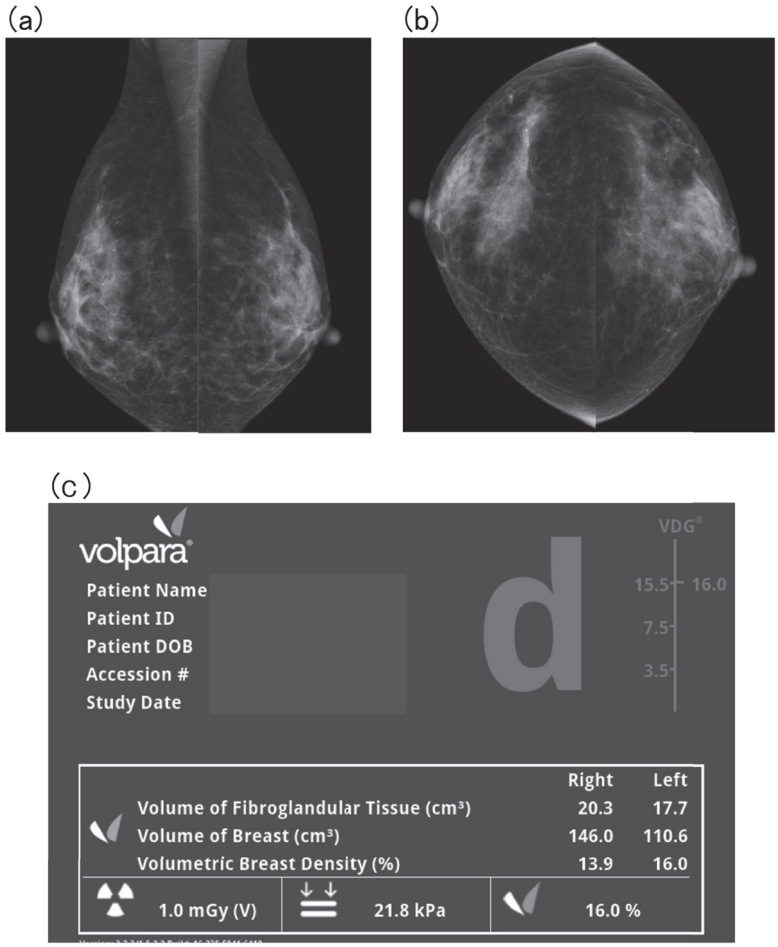

Fig.1. MLO (a) and CC (b) Views of Mammography that Showed Disagreement in Breast Density Category. Automated Breast Density (c) Assigned Grade d, but Radiologist Read Mammography as Grade 2

Table 5. Frequency of Breast Density Categories as Assessed by Two Radiologists

Radiologist 1 / Radiologist 2	1	2	3	4	total
1	39	24	0	0	63
2	5	656	131	0	792
3	0	127	456	16	599
4	0	0	0	2	2
total	44	807	587	18	1456

The agreement was good. (observed agreement 79%, κ values=0.701)

Table 6. Cross Tabulation between Visual Category and VDG

Volpara / Radiologist	Fatty (a, b)	Dense (c, d)	total
Fatty (1, 2)	189	505	694
Dense (3 ,4)	3	455	458
total	192	960	1152

There was fair agreement between visual assessment and VDG. (observed agreement 56%, κ value=0.224)

Fatty group (n=505, disagreement group) with the Dense group (n=455, agreement group).

Univariate analysis

Table 7 shows the results from univariate analysis. Age, menopause stage, difference in bilateral breast density, fibroglandular tissue volume, breast volume, and VBD were significantly different between the agreement and disagreement groups. Mammographic final assessment was the only variable without a significant difference.

Multivariate analysis

Table 8 shows the results from multivariate analysis. Of the factors included, VBD was an independent correlative factor with a greater possibility of significant disagreement (Odds ratio = 0.755; 95% CI, 0.730 – 0.782; p<0.05). Patient age (Odds ratio = 0.976; 95% CI, 0.947 – 1.006; p=0.122), menopause stage (Odds ratio=1.232; 95% CI, 0.731 – 2.077; p=0.433), and difference in bilateral breast density (Odds ratio=1.248; 95% CI, 0.789 – 1.974; p=0.343) were not significantly associated with disagreement.

Discussion

In the present study, we analyzed mammographic density in a screening population by comparing fully automated volumetric assessment using Volpara software with radiologists' assessments in Japanese women. We found that inter-observer agreement was good, but agreement between the radiologists and fully automated assessment was just fair.

Consistency between radiologist assessment and automated assessment has been controversial. Morissh *et al.*[15] found a weak correlation between Volpara estimates and observers' visual assessments. However, several other studies have noted moderate correlations[16,17]. In the report of the Japanese Dense Breast Correspondence Working Group, the proportion of dense breasts in Japan was estimated to be approximately 40% (unpublished date). This was similar to our radiologists' evaluations. In Korea, even though it is in the same region of Asia as Japan, Lee *et al.*[16] reported that 87% were dense breasts, by visual assessment and thus, there is a possibility of a considerable difference in assessment of mammographic density between countries. In addition to ethnicity and age group, many factors are thought to influence visual evaluation, such as the type of imaging apparatus, monitor, and positioning.

There are also differences in the rates of categories determine by Volpara among studies. In addition, in a large-scale study, Sartor *et al.* (Sweden)[17] showed that the proportion of dense breasts by automated quantitative estimates was 43% and Brandt *et al.* (USA)[18] showed that it was 51%. In our study, 85% of cases were assessed as dense breasts by Volpara. The percentage of dense breasts according to Volpara also markedly differs by country and race. In the present study, median breast volume was 327.2 cm^3, median fibroglandular tissue volume was 51.1 cm^3, and median VBD was 15.2%. Sartor *et al.* (Sweden)[17] found that median breast volume was 691.1 cm^3, median fibroglandular tissue volume was 49.0 cm^3, and median VBD was 7.2%. Among these findings, median fibroglandular tissue volume in our study did not substantially differ from those in the previous studies; however median

Table 7. Factors Affecting the Difference between the Agreement Group and the Disagreement Group (univariate analysis)

Variables	Agreement (n=455)	Disagreement(n=505)	*p* value
Age mean, years (SD)	49.2 (8.2)	52.5 (8.7)	p<0.05
Menopause stage Postmenopausal (%)	152 (33)	260 (51)	p<0.05
Difference in bilateral Breast density Difference (%)	39 (9)	97 (19)	p<0.05
Final assessment negative (%)	445 (98)	495 (98)	p=0.814
Fibroglandular tissue volume median, cm^3 (range)	59.0 (4.8 – 274.3)	39.1 (3.3 – 196.1)	p<0.05
Breast tissue volume median, cm^3 (range)	271.9 (14.7 – 1078.4)	331.3 (8.4 – 1216.8)	p<0.05
VBD median, % (range)	22.3 (7.9 – 42.0)	12.1 (5.0 – 29.5)	p<0.05

Table 8. Factors Affecting the Difference between Agreement Group and Disagreement Group (multivariate analysis)

Variables	Odds ratio (95% CI)	*p* value
Age	0.976 (0.947 – 1.006)	p =0.122
Menopause stage Premenopausal Postmenopausal	1.232 (0.731 – 2.077)	p =0.433
Difference in bilateral Breast density none Difference	1.248 (0.789 – 1.974)	p =0.343
VBD median	0.755 (0.730 – 0.782)	p <0.05

breast volume was approximately half of that in Sartor's results, which were all for European subjects. Since VBD is obtained by dividing fibroglandular tissue volume by breast volume, it is dependent on breast volume. Japanese women's VBD might be higher because their breast volume is small compared with European women. It is possible that more Japanese women would be determined to have dense breasts by Volpara and this might be a cause of poor agreement between it and visual assessment methods.

In our study, we extracted cases evaluated as dense breasts by Volpara and investigated factors associated with disagreement between it and visual assessment. In the disagreement group, median VBD was significantly lower than in the agreement group. Han *et al.*[16] showed that a difference in bilateral breast density can affect disagreement between a radiologist and automated software. Also, Seo *et al.*[19] showed that VBD was significantly lower in the disagreement group, as in our study. The authors of these studies explained that it may be difficult to evaluate scattered, small volumes of tissue visually.

The VBD calculated by Volpara software is the percentage of the fibroglandular tissue relative to the entire breast but unlike visual assessment, the possibility of lesions being masked is not mentioned in classification. We think this might be another factor behind the difference between visual assessment and Volpara.

In this study, regarding cases assessed as dense breasts according to Volpara, it is possible that those with lower a VBD would not be determined to be dense breasts in radiologists' assessments. To eliminate this divergence and to raise the coincidence rate between radiologists' visual assessments and Volpara estimates, further examination of cutoff values for dense breasts when using VBD in Japanese women may be necessary.

To overcome the masking of lesions, in Japan, it is recommended to use ultrasonography in addition to mammography for dense breasts. Objective and reproducible assessments for dense breasts is an urgent matter to be addressed, and interest in fully automated volumetric methods of measuring breast density is increasing. However, masking of lesions and volumetric breast density measurement according to Volpara estimates may not necessarily be correlated, so it may be premature to add ultrasound examination in all cases when breasts are evaluated by as dense by Volpara.

Some limitations of this study require consideration. First, only two radiologists determined the breast categories. Further investigation with additional readers is needed in order to apply Volpara estimates in a practical manner in the screening of Japanese women. Second, in this study, we classified breast density according to the Japanese categories. These categories are based on BI-RADS, which was drawn up by an American expert group of radiologists; however, the 2 sets of categories do not fully correspond with each other. Therefore, a comparison between our findings and those in Western countries is not necessarily accurate. Finally, some factors known to affect mammographic density - for example, the use of hormone replacement therapy or lactation period - were not considered. Based on these factors, further examination is necessary.

In conclusion, automated volumetric breast density measurement has fair agreement with radiologists' visual assessments. Low VBD was a factor associated with this difference. Although the use of a fully automated breast density measuring device would be effective due to its reproducibility and objectivity, the masking of lesions and volumetric breast density measured by software are not necessarily correlated and thus further research is necessary.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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(Received June 28, 2018 ; Accepted October 16, 2018)

Original Article

Ningen Dock International 2019 ; 6 : 44–49

High Blood Pressure a Risk Factor of Incident Ocular Hypertension in Japanese Men and Women Undergoing Health Screening

Eiji Oda

Abstract

Background and Aim: No longitudinal study has been conducted on risk factors of incident ocular hypertension (OH). The aim of this study was to find risk factors of OH defined as intraocular pressure (IOP) of ≥ 21 mmHg for one or both eyes in a health screening population.

Methods: This was a retrospective 8-year follow-up study of 1,950 men and 1,109 women whose IOPs were < 21 mmHg at baseline. Hazard ratios (HRs) of incident OH for candidate numerical and candidate categorical risk factors were calculated with Cox regressions.

Results: OH developed in 36 men (1.9%) and 12 women (1.1%) during the mean follow-up period of 6.1 years. Among numerical variables, an increase in systolic and diastolic blood pressures (BPs) and a decrease in HDL cholesterol were significant risk factors of OH in all participants. When calculated separately by gender, increases in systolic and diastolic BPs were significant risk factors of incident OH in men while an increase in diastolic BP was a significant risk factor of incident OH in women. Among categorical variables, hypertension and hypo-HDL cholesterolemia were significant risk factors of incident OH in all participants. When calculated separately by gender, hypertension and absence of daily alcohol drinking were significant risk factors of incident OH in men while no significant risk factor was found in women.

Conclusion: High BP was a significant risk factor of incident OH.

Keywords intraocular pressure, ocular hypertension, blood pressure, glaucoma

Glaucoma is the major cause of blindness. In 2013, the number of people aged 40–80 years with glaucoma worldwide was estimated to be 64.3 million and forecasted to increase to 76.0 million in 2020 and 111.8 million in 2040, disproportionally affecting people residing in Asia and Africa[1]. Age,[2–5] family history of glaucoma[6], thinner central cornea[7], myopia[8,9] and corticosteroid use [10] are considered to be risk factors of glaucoma. Some studies indicate that diabetes[11,12] and hypertension[13] may increase the risk of developing glaucoma. However, the most important risk factor of glaucoma is ocular hypertension (OH)[6,7,14].

Baseline intraocular pressure (IOP) increased disease progression and reduction of IOP by treatment was a major preventive factor of disease progression in the Early Manifest Glaucoma Trial[14]. Each one mmHg higher of IOP on follow-up was associated with an approximate 10% increased risk of disease progression[14]. Also, cross-sectional associations between IOP and systolic and diastolic blood pressures (BPs) and body mass index (BMI) were reported[15,16]. However, these cross-sectional associations cannot clarify cause-effect relationships. Longitudinal studies regarding incident open angle glaucoma (OAG) found that baseline hypertension and ocular perfusion pressure (OPP), defined as BP minus IOP, were negatively associated with incident OAG[17]. However, there has been no longitudinal study on risk factors of incident OH.

The aim of the present study was to find risk factors of incident OH in a health screening population. It was approved by the ethics committee of Tachikawa General Hospital and was performed in accordance with the ethical standards of the Declaration of Helsinki in 1964 and its later amendments.

Subjects and Methods

Subjects

Among 3,866 individuals who visited our Medical Check-up Center for general health screening between April 2008 and March 2009 and gave written informed consent to use their data for epidemiological studies, 3,839 individuals (63% men) aged 19–82 years completed ocular tonometry. They were all required to fill out a questionnaire recommended by the Japanese Min-

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istry of Health, Labour and Welfare including questions on histories of coronary heart disease and stroke, smoking and drinking status, physical activity, antihypertensive, antidiabetic, and antihyperlipidemic medications. After excluding individuals with IOP ≥ 21 mmHg for one or both eyes, 3,799 individuals remained as potential study participants. Owing to 740 individuals (19.5%) dropping out during the 8-year follow-up period, 3,059 individuals (63.7% men) aged 24–82 years were included in the present study.

Measurements

After an overnight fast, blood samples were obtained to measure blood levels of routine health screening parameters including fasting plasma glucose (FPG), triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, hemoglobin A1c (HbA1c), uric acid and hemoglobin. Chemical measurements were performed at BML Nagaoka (Nagaoka, Japan) by routine laboratory methods. LDL cholesterol was measured using a direct surfactant method with Choletest-LDL (Sekisui Medical Inc, Tokyo, Japan). HbA1c was measured by latex aggregation immunoassay using Determiner HbA1c (Kyowa Medex, Tokyo, Japan) and expressed in NGSP%. IOP was measured by a trained clinical technician using Full Auto Tonometer TX-20P (Canon, Tokyo, Japan). Average systolic BPs and diastolic BPs were calculated from two automatic measurements using a MPV-3301 device (NIHON KOHDEN CORPORATION, Tokyo, Japan) in the sitting position after a 5 min rest period. Body height and weight were automatically measured using a TBF-210 device (TANITA corporation, Tokyo, Japan) wearing the light clothing provided by our center whose weight was subtracted from the measured body weight. BMI was calculated as weight in kilograms divided by the square of height in meters.

Definition of candidate categorical risk factors

Candidate categorical risk factors were defined as below.
Obesity: BMI ≥ 25 kg/m^2.
Hypertension: systolic BP ≥ 140 mmHg or diastolic BP ≤ 90 mmHg or use of antihypertensive drugs.
Diabetes: FPG ≥ 7.0 mmol/L or HbA1c ≥ 6.5% or use of antidiabetic drugs.
Hypercholesterolemia: total cholesterol ≥ 6.2 mmol/L or use of antihyperlipidemic drugs.
Hyper-LDL cholesterolemia: LDL cholesterol ≥ 3.6 mmol/L or use of antihyperlipidemic drugs.
Hypertriglyceridemia: triglycerides ≥ 1.7 mmol/L.
Hypo-HDL cholesterolemia: HDL cholesterol < 1.0 mmol/L in men and < 1.3 mmol/L in women.
Hyperuricemia: uric acid ≥ 400 μmol/L in men and ≥ 360 μmol/L in women.
Anemia: hemoglobin < 130 g/L in men and < 120 g/L in women.

Hypo-HDL cholesterolemia was defined differently by sex according to the international definition[18], although the definition for men and women is the same in Japan. Detailed information on antihyperlipidemic drugs was not available but most of the antihyperlipidemic drugs were thought to be prescribed for hypercholesterolemia. Therefore, antihyperlipidemic drugs were all considered to be antihypercholesterolemic drugs in this study. This assumption is not exactly scientific but was a practical way of dealing with the limited information.

Statistical analysis

Baseline variables were compared between participants who developed OH during the follow-up period and those who did not. In this study, the information available on alcohol consumption was only that pertaining to daily alcohol drinking. Physical activity was defined as walking for one hour or longer per day or exercising for 30 minutes or longer twice or more per week. Means were compared by *t*-tests and percentages were compared by chi-squared tests.

Hazard ratios (HRs) of OH were calculated using Cox regression models in which years were used as the unit of the survival variable, the first diagnosis with OH by annual ocular tonometry was ascertained as the outcome and subjects without the outcome were censored at their last annual health screening visit. Firstly, sex- and age-adjusted HRs for numerical variables and current smoking, daily alcohol drinking and physical activity were calculated. The HRs were calculated for each one unit increase for numerical variables except for BPs. For BPs, the HRs were calculated for each 10 mmHg increase. Then, sex- and age-adjusted multivariable HRs were calculated for variables with $p < 0.1$. The multivariable HRs were separately calculated for systolic BP and diastolic BP because these BPs were strongly correlated ($r = 0.93$). Age-adjusted HRs and age-adjusted multivariable HRs were also calculated separately by gender.

Secondly, sex- and age-adjusted HRs were calculated for current smoking, daily alcohol drinking, physical activity, history of stroke, history of coronary heart disease and the candidate categorical risk factors defined above. Then, sex- and age-adjusted multivariable HRs were calculated for variables with $p < 0.1$. Age-adjusted HRs and age-adjusted multivariable HRs were also calculated separately by gender. Statistical analyses were performed using Dr SPSS-2 (IBM Japan, Tokyo, Japan). *p* values of lower than 0.05 were considered significant.

Results

During the 8-year follow-up period, OH developed in 36 men (1.9%) and 12 women (1.1%). The mean follow-up period was 6.1 years.

Baseline data stratified by development status of OH

Table 1. Baseline Data Stratified by Development Status of Ocular Hypertension

	developers	non-developers	p [h]
n	48	3011	
men (%)	75.0	63.6	0.102
age (years)	52.5 (9.3)	51.8 (9.4)	0.608
right intraocular pressure (mmHg)	17.1 (2.9)	13.0 (2.7)	<0.001
left intraocular pressure (mmHg)	17.0 (2.6)	13.0 (2.7)	<0.001
body mass index (kg/m^2)	23.3 (3.3)	22.7 (3.0)	0.138
systolic blood pressure (mmHg)	126.0 (19.8)	118.5 (17.5)	0.003
diastolic blood pressure (mmHg)	79.9 (11.7)	74.8 (11.0)	0.001
fasting plasma glucose (mmol/L)	5.4 (0.9)	5.2 (0.8)	0.043
HbA1c (%)	5.6 (0.6)	5.4 (0.5)	0.058
total cholesterol (mmol/L)	5.23 (0.90)	5.29 (0.81)	0.655
LDL cholesterol (mmol/L)	3.20 (0.73)	31.4 (0.75)	0.574
HDL cholesterol (mmol/L)	1.44 (0.36)	1.59 (0.40)	0.009
triglycerides (mmol/L)	1.43 (0.98)	1.21 (0.79)	0.055
uric acid (μmol/L)	354 (95)	328 (83)	0.030
hemoglobin (g/L)	145 (15)	142 (15)	0.070
antihypertensive drugs (%)	31.3	16.1	0.005
antidiabetic drugs (%)	6.3	2.5	0.096
antihyperlipidemic drugs (%)	12.5	10.1	0.590
history of stroke (%)	0.0	1.4	0.416
history of coronary heart disease (%)	4.2	3.3	0.746
current smoking (%)	25.0	23.9	0.865
daily alcohol drinking (%)	31.3	38.2	0.323
physical activity [a] (%)	31.3	35.7	0.526
obesity [b] (%)	29.2	20.2	0.125
hypertension (%)	47.9	25.3	<0.001
diabetes (%)	8.3	4.2	0.157
hypercholesterolemia [c] (%)	25.0	22.1	0.629
hyper-LDL cholesterolemia [d] (%)	31.3	33.2	0.775
hypo-HDL cholesterolemia [e] (%)	16.7	7.9	0.026
hypertriglyceridemia (%)	27.1	17.1	0.070
hyperuricemia [f] (%)	25.0	17.4	0.170
anemia [g] (%)	6.3	8.0	0.651

mean (SD) or %, [a] walking for one hour or longer per day or exercising for 30 minutes or longer twice or more per week, [b] BMI ≥ 25 kg/m^2, [c] total cholesterol ≥ 6.2 mmol/L or use of antihyperlipidemic drugs, [d] LDL cholesterol ≥ 3.6 mmol/L or use of antihyperlipidemic drugs, [e] HDL cholesterol < 1.0 mmol/L in men and < 1.3 mmol/L in women, [f] uric acid ≥ 400 μ mol/L in men and ≥ 360 μ mol/L in women, [g] hemoglobin < 130 g/L in men and < 120 g/L in women, [h] *t*-tests for means and chi-squared tests for percentages

are presented in **Table 1**. IOP, systolic BP, diastolic BP, FPG, and uric acid were significantly higher and HDL cholesterol was significantly lower in OH developers than non-developers. The prevalences of hypertension and hypo-HDL cholesterolemia were significantly higher in OH developers than non-developers.

The HRs (95% confidence intervals (CIs)) of incident OH for candidate numerical risk factors are shown in **Table 2**. The sex- and age-adjusted HRs were significant for systolic BP, diastolic BP and HDL cholesterol in all participants. When the age-adjusted HRs were calculated separately by gender, they were significant for systolic BP, diastolic BP and HDL cholesterol in men while only the HR for diastolic BP was significant in women. In the multivariable analysis, systolic BP and diastolic BP were significantly positively associated with incident OH while HDL cholesterol was marginally negatively associated with incident OH in all participants. In the gender-stratified analysis, systolic BP and diastolic BP were significantly positively associated with incident OH in men while diastolic BP was significantly positively associated with incident OH in women.

The HRs (95% CIs) of incident OH for candidate categorical risk factors are shown in **Table 3**. The sex- and age-adjusted HRs for hypertension and hypo-HDL cholesterolemia were significant in all participants. When analyzed separately by gender, the age-adjusted HR for hypertension was significant in men. In women, while no significant risk factor was found, hypo-HDL cholesterolemia was marginally associated with incident OH. In multivariable analysis, hypertension and hypo-HDL cholesterolemia were significantly associated with inci-

Table 2. Hazard Ratios of Incident Ocular Hypertension for Numerical Variables

	all participants		men		women	
	HR [e] (95% CI [f])	*p*	HR [e] (95% CI [f])	*p*	HR [e] (95% CI [f])	*p*
sex and age adjusted hazard ratios						
body mass index, kg/m^2	1.05 (0.96 – 1.15)	0.266	1.08 (0.97 – 1.20)	0.162	1.00 (0.83 – 1.19)	0.963
systolic BP [a], 10 mmHg	1.22 (1.05 – 1.43)	0.009	1.22 (1.02 – 1.46)	0.028	1.25 (0.93 – 1.70)	0.143
diastolic BP [a], 10 mmHg	1.43 (1.11 – 1.84)	0.006	1.37 (1.02 – 1.84)	0.039	1.64 (1.03 – 2.60)	0.038
ocular PP [b], 10 mmHg	1.06 (0.84 – 1.34)	0.643	1.04 (0.80 – 1.36)	0.759	1.12 (0.70 – 1.80)	0.640
FPG [c], mmol/L	1.22 (0.96 – 1.56)	0.101	1.23 (0.96 – 1.58)	0.102	1.12 (0.47 – 2.67)	0.793
HbA1c, %	1.40 (0.97 – 2.04)	0.075	1.39 (0.93 – 2.07)	0.110	1.66 (0.47 – 5.93)	0.432
total cholesterol, mmol/L	0.93 (0.65 – 1.34)	0.707	0.87 (0.57 – 1.32)	0.508	1.19 (0.61 – 2.33)	0.602
LDL cholesterol, mmol/L	1.12 (0.77 – 1.63)	0.558	0.99 (0.63 – 1.55)	0.964	1.61 (0.83 – 3.12)	0.158
HDL cholesterol, mmol/L	0.36 (0.15 – 0.86)	0.021	0.35 (0.13 – 0.98)	0.046	0.36 (0.07 – 1.97)	0.241
triglycerides, mmol/L	1.20 (0.95 – 1.53)	0.127	1.23 (0.99 – 1.54)	0.063	0.52 (0.10 – 2.66)	0.433
uric acid, μ mol/L	1.00 (1.00 – 1.01)	0.118	1.00 (1.00 – 1.01)	0.107	1.00 (0.99 – 1.01)	0.680
hemoglobin, g/L	1.01 (0.99 – 1.04)	0.314	1.01 (0.98 – 1.05)	0.371	1.02 (0.97 – 1.07)	0.507
current smoking	0.95 (0.48 – 1.87)	0.874	1.05 (0.52 – 2.12)	0.883	diverged	0.979
daily alcohol drinking	0.57 (0.30 – 1.09)	0.087	0.51 (0.26 – 1.00)	0.050	1.17 (0.25 – 5.36)	0.841
physical activity [d]	0.81 (0.44 – 1.50)	0.507	1.01 (0.51 – 2.01)	0.971	0.35 (0.08 – 1.62)	0.181
sex and age adjusted multivariable hazard ratios including covariates with $p < 0.1$						
systolic BP [a], 10 mmHg	1.21 (1.04 – 1.41)	0.014	1.21 (1.01 – 1.44)	0.036		
HDL cholesterol, mmol/L	0.44 (0.18 – 1.08)	0.073	0.52 (0.17 – 1.58)	0.251		
HbA1c, %	1.25 (0.83 – 1.88)	0.282				
triglycerides, mmol/L			1.15 (0.86 – 1.55)	0.351		
daily alcohol drinking	0.65 (0.34 – 1.25)	0.196	0.53 (0.26 – 1.08)	0.082		
sex and age adjusted multivariable hazard ratios including covariates with $p < 0.1$						
diastolic BP [a], 10 mmHg	1.43 (1.13 – 1.82)	0.003	1.36 (1.01 – 1.82)	0.042	1.64 (1.03 – 2.60)	0.038
HDL cholesterol, mmol/L	0.41 (0.18 – 0.95)	0.038	0.53 (0.17 – 1.60)	0.260		
HbA1c, %	1.26 (0.84 – 1.87)	0.262				
triglycerides, mmol/L			1.16 (0.86 – 1.57)	0.339		
daily alcohol drinking	0.69 (0.37 – 1.27)	0.230	0.52 (0.26 – 1.06)	0.073		

[a] blood pressure, [b] mean blood pressure minus mean intraocular pressure, [c] fasting plasma glucose, [d] walking for one hour or longer per day or exercising for 30 minutes or longer twice or more per week, [e] hazard ratio, [f] confidence interval. Blank cells in the table indicate that *p* for the covariate in the left column is higher than 0.1.

dent OH in all participants. When analyzed separately by gender, hypertension was positively associated with incident OH and daily alcohol drinking was negatively associated with incident OH in men, and in women, while there was no significant risk factor, hypo-HDL cholesterolemia was marginally associated with incident OH.

Discussion

The present 8-year follow-up study found that hypertension, hypo-HDL cholesterolemia, increase in systolic and diastolic BPs and decrease in HDL cholesterol were significant risk factors of incident OH in all participants. When calculations were made separately by gender, hypertension, increase in systolic and diastolic BPs and absence of daily alcohol drinking were significant risk factors in men while only increase in diastolic BP was a significant risk factor in women. Although baseline IOP may be the strongest risk factor of incident OH, it was excluded from the risk factors in the present study.

The association between BP and OH is controversial. Seddon *et al.* reported in a case-control study that the variables independently associated with OH were family history of glaucoma, myopia, absence of alcohol intake, history of non-ocular surgery, high income and history of hypertension[13]. The Beaver Dam Eye Study reported that IOP was associated with systolic and diastolic BPs, BMI, hematocrit, glycosylated hemoglobin, cholesterol, pulse, nuclear sclerosis, season, and time of day of measurement[15]. In a large-scale population of ophthalmologically normal Japanese subjects, IOP was negatively correlated with age, corneal radius and refraction but positively correlated with central corneal thickness, systolic BP and BMI[16]. In the present longitudinal study, increase in BP was a significant risk factor of incident OH, whereas BMI, hemoglobin, FPG, HbA1c, total cholesterol and LDL cholesterol were not significantly associated with incident OH. Ponte *et al.* performed a case-control study as a part of the Casteldaccia Eye Study to investigate risk factors of OH and glaucoma, and showed that only use of ocular corti-

Table 3. Hazard Ratios of Incident Ocular Hypertension for Categorical Variables

	all participants		men		women	
	HR [i] (95% CI [j])	*p*	HR [i] (95% CI [j])	*p*	HR [i] (95% CI [j])	*p*
sex- and age-adjusted hazard ratios						
history of stroke	diverged	0.972	diverged	0.973	diverged	0.985
history of CHD [a]	1.10 (0.26 – 4.64)	0.899	1.21 (0.28 – 5.18)	0.800	diverged	0.984
current smoking	0.95 (0.48 – 1.87)	0.874	1.05 (0.52 – 2.12)	0.883	diverged	0.979
daily alcohol drinking	0.57 (0.30 – 1.09)	0.087	0.51 (0.26 – 1.00)	0.050	1.17 (0.25 – 5.36)	0.841
physical activity [b]	0.81 (0.44 – 1.50)	0.507	1.01 (0.51 – 2.01)	0.971	0.38 (0.08 – 1.79)	0.223
obesity [c]	1.51 (0.81 – 2.82)	0.198	1.40 (0.69 – 2.85)	0.350	2.02 (0.54 – 7.48)	0.295
hypertension	2.70 (1.47 – 4.98)	0.001	2.89 (1.44 – 5.77)	0.003	2.08 (0.51 – 8.47)	0.306
diabetes	1.87 (0.66 – 5.32)	0.241	2.01 (0.70 – 5.79)	0.197	diverged	0.985
hypercholesterolemia [d]	1.21 (0.62 – 2.35)	0.576	0.97 (0.43 – 2.23)	0.949	2.30 (0.67 – 7.98)	0.188
hyper-LDLC [e]	0.91 (0.49 – 1.69)	0.772	0.80 (0.39 – 1.66)	0.550	1.47 (0.44 – 4.92)	0.536
hypo-HDLC [f]	2.49 (1.16 – 5.32)	0.019	2.12 (0.82 – 5.44)	0.120	3.64 (0.98 – 13.44)	0.053
hypertriglyceridemia	1.61 (0.84 – 3.10)	0.153	1.87 (0.94 – 3.69)	0.073	diverged	0.981
hyperuricemia [g]	1.43 (0.73 – 2.81)	0.294	1.45 (0.71 – 2.97)	0.303	1.50 (0.19 – 11.83)	0.699
anemia [h]	0.91 (0.27 – 3.01)	0.876	0.74 (0.10 – 5.48)	0.766	1.04 (0.21 – 5.06)	0.963
sex- and age-adjusted multivariable hazard ratios including covariates with $p < 0.1$						
hypertension	2.74 (1.49 – 5.02)	0.001	2.83 (1.42 – 5.67)	0.003		
hypo-HDLC [f]	2.31 (1.07 – 4.99)	0.032			3.64 (0.98 – 13.44)	0.053
hypertriglyceridemia			1.71 (0.87 – 3.39)	0.122		
daily alcohol drinking	0.61 (0.32 – 1.16)	0.130	0.49 (0.25 – 0.98)	0.043		

[a] coronary heart disease, [b] walking for one hour or longer per day or exercising for 30 minutes or longer twice or more per week, [c] BMI ≥ 25 kg/m^2, [d] total cholesterol ≥ 6.2 mmol/L or use of antihyperlipidemic drugs, [e] LDL cholesterol ≥ 3.6 mmol/L or use of antihyperlipidemic drugs, [f] HDL cholesterol < 1.0 mmol/L in men and < 1.3 mmol/L in women, [g] uric acid ≥ 400 μ mol/L in men and ≥ 360 μ mol/L in women, [h] hemoglobin < 130 g/L in men and < 120 g/L in women, [i] hazard ratio, j confidence interval. Blank cells in the table indicate that *p* for the covariate in the left column is higher than 0.1.

costeroids and myopia were independently associated with OH or glaucoma[10]. However, these previous studies were all cross-sectional.

Regarding risk factors of OAG, Gordon *et al*. reported that independent risk factors were older age, larger vertical or horizontal cup-disc ratio, higher IOP, greater pattern standard deviation and thinner central corneal thickness in the Ocular Hypertension Treatment Study[7]. Leske *et al*. reported that the risk of OAG substantially increased as baseline IOP increased, while persons with hypertension at baseline had half the risk of OAG, suggesting that a lower OPP at baseline increased the risk of OAG[17]. Satilmis *et al*. examined a correlation between progression rate of glaucomatous damage and retrobulbar blood flow in 20 OAG patients and reported that rate of progression of glaucomatous damage was negatively correlated with baseline end-diastolic blood flow velocity in the central retinal artery and positively correlated with baseline IOP[19]. This finding may partly explain the negative association between baseline BP and incident OAG.

The present longitudinal study observed that baseline BP and hypertension were positively associated with incident OH. Klein *et al*. investigated a relationship between change in BPs and change in IOP and reported that IOP was significantly positively correlated with systolic and diastolic BPs at both baseline and 5-year follow-up and there were significant direct correlations between changes in BPs and changes in IOP[20]. The findings of this study support the results of the present study.

An increase in BP may elevate ciliary arterial BP resulting in an increase in aqueous humor production and IOP elevation[21]. Although a low BP may lower OPP and increase the likelihood of ischemic stress on the optic nerve, hypertension may increase risk via IOP elevation and hypertensive vascular injury[22]. Influenced by the autoregulatory capacity of the eye, the balance between IOP and BP may be a factor determining whether an individual will develop optic nerve damage[22].

The present study suggested that hypo-HDL cholesterolemia or a decrease in HDL cholesterol may be a risk factor of incident OH. However, the association became insignificant when analyzed separately by gender. Further studies are required regarding the association between HDL cholesterol and incident OH.

Limitations

The present study was a retrospective follow-up study. The participants were not recruited from a general population and detailed information on ophthalmological diseases and IOP-related medications, demographic backgrounds and medications was not available. There-

fore, residual confounders might have influenced the present results. The number of female participants was too small to detect significant associations between incident OH and risk factors other than increase in diastolic BP.

Conclusions

The present 8-year follow-up study found that hypertension, hypo-HDL cholesterolemia, increase in systolic and diastolic BPs and decrease in HDL cholesterol were significant risk factors of incident OH in all participants. Hypertension, increase in systolic and diastolic BPs and absence of daily alcohol drinking were significant risk factors in men while only increase in diastolic BP was a significant risk factor in women when calculations were made separately by gender. The number of the female participants in the present study was too small to detect significant associations.

Acknowledgments

The author thanks all subjects who participated in the study and the paramedical staff at our center who assisted with it. The sole author, Oda E, received no financial support and has no conflict of interest.

Conflict of Interest

none.

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(Received September 11, 2018 ; Accepted November 13, 2018)

Original Article

Ningen Dock International 2019 ; 6 : 50–55

*Helicobacter pylori A*ntibody Titers in the High Normal Range Include Cases of *H. pylori* Infection in Patients with *H. pylori*-related Gastritis

Kyoko Ito[1], Tomohiro Kato[1,2]

Abstract

Objective: *Helicobacter pylori* (Hp) infection plays a critical role in gastric neoplasm pathology. Detecting Hp and Hp-related gastric diseases is important for gastric cancer prevention. Recent studies showed that Hp infection was missed to be diagnosed in some patients with atrophic gastritis when serum anti-Hp antibody enzyme immunoassay detection tests (Hp tests) were used. Here, we review the results of Hp testing over a 5-year period in patients with Hp-related gastritis.

Methods: We included 4,319 subjects who underwent optional Hp testing at our institute during Ningen Dock from 2012 to 2016. We investigated the prevalence of Hp positivity in spite of technically negative antibody titers of 3-9 (high negative) in Hp tests in Hp-related gastritis. Single and multiple logistic regression analyses were performed to identify factors associated with Hp infection among a total of 26 potential factors.

Results: The prevalence rates of positive and high negative Hp results were 31.6% and 11.4%, respectively, with no differences between the sexes. In patients with Hp-related gastritis, the prevalence rates of positive and high negative Hp results were 62.5% and 14.2%, respectively. Age, anemia, increased white blood cell count, and higher aspartate aminotransferase (AST) levels were associated with Hp infection ($p < 0.05$).

Conclusions: We identified possible Hp infection in patients with Hp-related gastritis who tested negative for serum anti-Hp antibodies. Anemia was a factor associated with Hp infection, which is consistent with previous studies. We also found that elevated AST levels were associated with Hp infection, a novel finding. Further studies are needed to clarify the pathology.

Keywords *Helicobacter pylori*, serum *Helicobacter pylori* antibody test, *Helicobacter pylori*-related gastritis, *Helicobacter pylori* infection-related factors

Helicobacter pylori (Hp) infection is known to be a major factor in gastric carcinogenesis[1]. In Japan, because of the high morbidity associated with gastric cancer, the government requires gastric cancer screening every year after the age of 40. Upper gastrointestinal series (UGI) or esophagogastroduodenoscopy (EGD) are the usual gastric cancer screening methods used but specific tests for detecting Hp infection may provide additional information for predicting future gastric cancers. At our facility, tests for detecting Hp infection have been offered as an optional additional examination. Since 2012, we have provided serum anti-Hp antibody testing (Hp test) for Hp detection since it is less invasive than other examinations. Here, we review the results of Hp testing at our facility over the last 5 years.

High negative serum anti-Hp antibody test results obtained using enzyme immunoassay (EIA) kits (Eiken Chemical Co., Ltd., Tokyo, Japan) have recently been shown to indicate Hp infection[2,3]. Patients with antibody titer results in the 3–9 range in the EIA test were later diagnosed as Hp infection in other examinations. We evaluated the actual rates of Hp detection in cases of Hp-related gastritis.

In addition, we identified factors associated with Hp infection using data from Ningen Dock medical evaluations. Hp infection is also related to diseases other than stomach disorders, such as anemia[4], atherosclerosis[5], diabetes mellitus (DM)[6], and idiopathic thrombocytopenic purpura (ITP)[7].

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Methods

We evaluated a total of 4,319 patients that underwent Hp testing in Ningen Dock, a medical check-up system unique to Japan in which patients are examined and undergo testing to aid early detection and prevention of latent diseases. We reviewed the results for patients seen at Center for Preventive Medicine of The Jikei University Hospital from 2012 to 2016. Informed consent was obtained from each subject, and this study was approved by the research ethics committee of The Jikei University School of Medicine. For patients who underwent multiple Hp tests, the data recorded at the first visits were used in the analyses. Patients who had already undergone Hp eradication therapy were excluded. The final number of subjects whose data were analyzed in our study was 2,668 (884 women, 1,784 men; mean age: 52.6 ± 11.0 years old; age range: 22–90).

Serum anti-*Helicobacter pylori* antibody testing

Fresh serum taken from each patient was examined using the anti-Hp test E-plate (Eiken Chemical Co., Ltd., Tokyo, Japan at SRL Inc., Tokyo, Japan). Antibody titers ≥ 10 were categorized as positive. Antibody titers <3 were categorized as negative. Antibody titers from 3 to 9 were categorized as high negative. Patients with high negative titers have been reported to include those with Hp infection[2,3].

Examinations of the upper GI tract

Esophagogastroduodenoscopy (EGD) was performed on-demand in 923 patients. Three independent expert endoscopists diagnosed these patients with atrophic gastritis or other forms of Hp-related gastritis according to the Kimura-Takemoto classification[8] or nodular gastritis[9]. Two medical imaging interpretation specialists and two expert gastroenterologists, who were board certified and had more than 10 years experience as a gastroenterologist, independently diagnosed gastritis in 948 subjects who underwent upper gastro-intestinal series (UGI) in the basic course of Ningen Dock examinations. Gastritis was diagnosed according to a textbook[10].

Investigation of factors associated with *Helicobacter pylori* infection

Twenty-six factors including age, sex, current smoking status, alcohol consumption (ethanol g/week), abdominal circumference, metabolic syndrome, liver function testing (serum aspartic aminotransferase (AST), alanine aminotransferase (ALT), and γ- glutamyl transpeptidase (γ-GTP) individually, and evidence of liver dysfunction [AST > 30 IU/L, ALT > 30 IU/L, or γ-GTP > 50 IU/L for men and > 30 IU/L for women]), fatty liver, lipid metabolism (serum total-cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) and triglyceride (TG) individually, and evidence of dyslipidemia [TC > 219 mg/dL, LDL-cholesterol > 139 mg/dL, HDL-C < 40 mg/dL or triglyceride (TG) > 149 mg/dL]), DM, glucose metabolism (fasting blood sugar (FBS), HbA1c and evidence of impaired glucose tolerance (IGT) [(fasting blood sugar (FBS) > 109 mg/dL or/and HbA1c > 5.6 % and insulin resistance (HOMA-IR = fasting serum insulin (μU/mL) x FBS (mg/dL)/405)), white blood cell count (WBC), platelet cell count (Plt), hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg), anemia (Hb <13.0 g/dL in men, Hb <12.0 g/dL in women), and serum iron (Fe) (μg/dL) were examined.

First, we performed a single logistic regression analysis to investigate a relationship between each of the factors and Hp infection. Since those with anti-Hp antibody titers of 3–9 have been reported to include both cases of Hp infection and non-infection[2,3], we excluded them. Next, we conducted a multiple logistic regression analysis to determine the factors that might predict Hp infection, on factors identified in the single logistic regression analysis as having a statistically significant relationship (defined as $p < 0.05$) with Hp infection. Odds ratios and 95% confidence intervals (CIs) are presented as measures of the associations. All statistical analyses were performed using SAS version 9.2 (SAS Institute Japan Ltd., Tokyo, Japan). $p < 0.05$ was considered statistically significant.

Results

Serum anti-*Helicobacter pylori* antibody EIA test

At our center, the Hp test is offered as an optional examination. Approximately 8,000 people undergo Ningen Dock every year at our facility, and approximately 10% opt for Hp testing. The annual difference in the number of patients undergoing Hp testing is shown in **Fig. 1**. The number of patients tested increased significantly every year, going from 372 patients in 2012, to 1,049 in 2014. From 2014 to 2016, the number remained steady, with approximately 1,050 patients tested in 2016. **Fig. 2** shows the age distribution of HP test examinees at approximately 1,000–1,500. Most were in the 40–50 years age range.

The prevalence of Hp was 31.6%, with no differences between the sexes (**Table 1**). High negative serum anti-Hp antibody titers, from 3–9, were obtained in 11.4% of patients. Although these patients were diagnosed as Hp-negative, they might possibly have had Hp infection. Among them there were no post Hp eradication cases, because they had been excluded. There was no difference between the sexes regarding the rate of high negative anti-Hp antibody titers. The prevalence of Hp increased with aging (**Fig. 3 (A)**). The highest prevalence, of 60.0%, was in patients in their 80s. High Hp prevalence was found in patients in their 20s. However, total number of examinees was too small for accurate

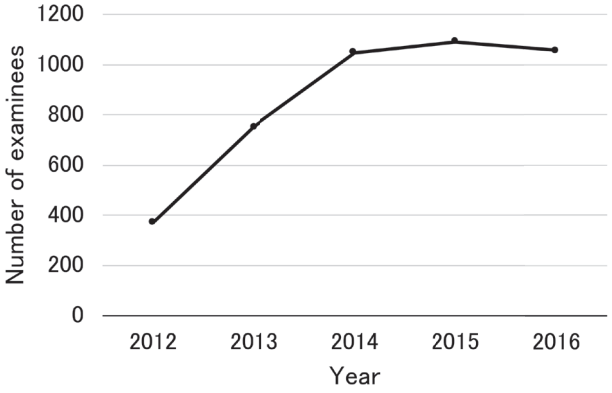

Fig.1. Number of Patients who Chose the Serum Anti-Hp Antibody Test each Year from 2012 to 2016

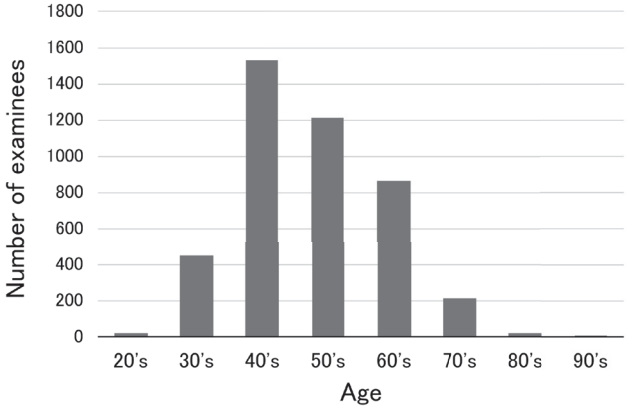

Fig.2. Total Number of Serum Anti-Hp Antibody Test Examinees by Patient Age

Table 1. Prevalence of Hp Infection

	Number of subjects	Prevalence of Hp infection (%)	Rate of high negative Hp antibody titers* (%)
Total	2668	31.6	11.4
Female	884	30.9	10.6
Male	1784	32.0	11.8

*3-9 Hp antibody titer

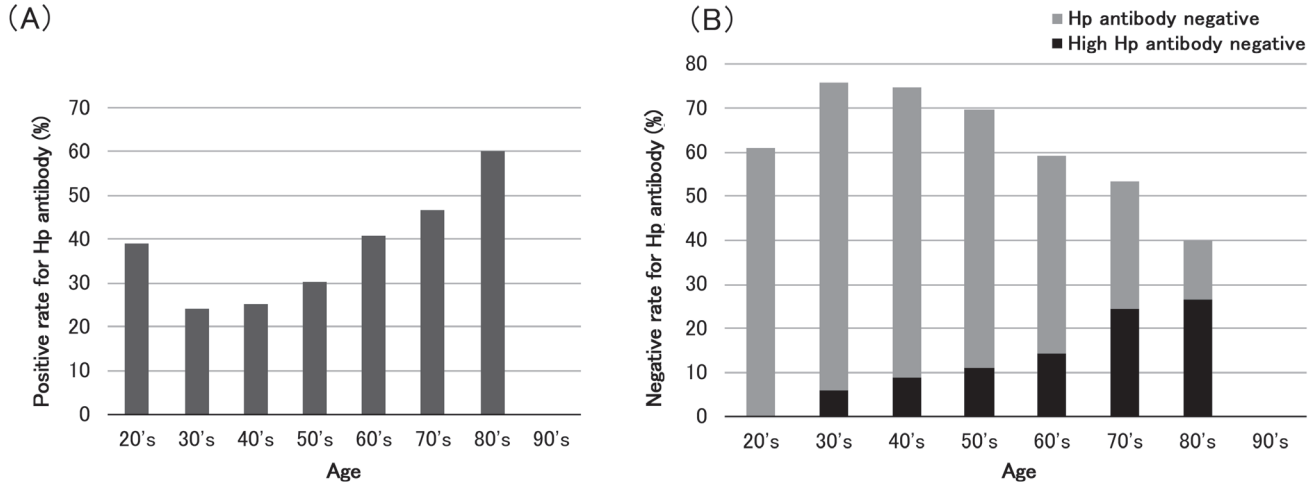

Fig.3. Age-related Rate of Hp-positive or Negative Diagnoses Via Serum Anti-Hp Antibody Testing

(A) Age-related rate of Hp-positive test results in the serum anti-Hp antibody test.

(B) Age-related rate of Hp-negative test results in the serum anti-Hp antibody test. The dark gray area in the bars shows the number of patients with high negative antibody titers (3-9) with an Hp-negative diagnosis.

analysis. The rate of high negative antibody titers also increased with aging (**Fig. 3 (B)**). Since the number of Hp-negative patients decreased with aging, the high negative Hp antibody titers accounted for a higher percentage of Hp-negative diagnoses in those of higher age. Thus, there is a high probability that Hp-positive patients included those who were diagnosed as Hp-negative based on the serum anti-Hp antibody test. This would have occurred more frequently in elderly people.

Anti-Hp antibody testing in Hp-related gastritis

Hp infection of the gastric mucosa induces forms of gastritis, such as atrophic[11] and nodular gastritis[12]. In our study, 360 (62.5%) out of 576 patients that suffered from Hp-related gastritis were Hp-positive (**Table**

Table 2a. Results of Hp Test in Hp-related Gastritis

		Number of Hp positive	Number of high Hp negative
Total		843 (31.6%)	305 (11.4%)
EGD	Total (*n*=923)	363 (39.3%)	103 (11.2%)
	Hp-related gastritis positive (*n*=576)	360 (62.5%)	82 (14.2%)
	Hp-related gastritis negative (*n*=347)	3 (0.9%)	21 (6.1%)
UGI	Total (*n*=948)	298 (31.4%)	96 (10.1%)
	Gastritis positive (*n*=442)	283 (64.0%)	62 (14.0%)
	Gastritis negative (*n*=506)	15 (3.0%)	34 (6.7%)

Table 2b. Endoscopic Findings in Stomach or Duodenum for Patients with High Normal Range Anti-Hp Antibody Titers

Endoscopic findings	Number of cases
Atrophic gastritis	82
Metaplastic change	2
Gastric ulcer	8
Gastric erosion	15
Gastric polyp	7
Gastric verrucosa	5
Submucosal tumor	2
Superficial gastritis	3
Angiodysplasia	2
Gastric diverticulum	1
Post gastrectomy	2
Duodenal ulcer	6
Duodenitis	4
Duodenal polyp	2
Duodenal diverticulum	1
Brunner's gland hyperplasia	1
Normal findings	7

Table 3. Single Logistic Regression Analysis

Factor	*p* value	Odds Ratio	95%CI
Age	<0.001*	1.043	1.034 – 1.051
Sex	0.421	1.076	0.900 – 1.297
Smoking	0.150	0.846	0.674 – 1.062
Alcohol consumption	0.378	1.000	1.000 – 1.001
Anemia	0.048*	1.347	1.003 – 1.811
Fe	0.392	0.999	0.997 – 1.001
WBC	0.001*	1.000	1.000 – 1.000
Plt	0.020*	0.998	0.996 – 1.000
HT	<0.001*	1.453	1.188 – 1.778
Dyslipidemia	0.471	1.067	0.895 – 1.271
TC	0.392	0.999	0.997 – 1.001
LDL cholesterol	0.177	1.002	0.999 – 1.005
HDL cholesterol	0.039*	0.995	0.990 – 1.000
TG	0.627	1.000	0.999 – 1.001
FBS	0.019*	1.006	1.001 – 1.011
HbA1c	0.009*	1.204	1.047 – 1.384
Insulin resistance	0.646	0.985	0.923 – 1.051
DM	0.057	1.452	0.989 – 2.130
Impaired glucose tolerance	<0.001*	1.433	1.166 – 1.761
AST	0.008*	1.012	1.003 – 1.021
ALT	0.278	1.003	0.998 – 1.008
γ -GTP	0.051	1.002	1.000 – 1.003
Liver dysfunction	0.684	1.037	0.870 – 1.236
Fatty liver	0.374	1.092	0.899 – 1.326
Abdominal circumference	0.117	1.007	0.998 – 1.016
Metabolic syndrome	0.040*	1.298	1.012 – 1.664

*Statistically significant. WBC: white blood cell count; Plt: platelet cell count; Fe: iron; HT: hypertension; TC: total cholesterol; LDL: low density lipoprotein; HDL high density lipoprotein; TG: triglyceride: FBS: fasting blood sugar; HbA1c: hemoglobin A1c; DM: diabetes mellitus; AST: aspartate aminotransferase; ALT: alanine aminotransferase; γ -GTP: γ -glutamyl transpeptidase; CI: confidence interval

2a) in the endoscopic examination. This result indicates that 37.5% of Hp-related gastritis cases were not due to Hp infection. However, among Hp-negative patients, we found that 14.2% had high negative antibody titers (titer range: 3–9). In a recent study in which patients with anti-Hp antibody titers of <10 were diagnosed as Hp-negative, among those with antibody titers in the range 3–9 in the EIA test, there were actually those with Hp infection. We found that 21 (6.1%) out of 347 subjects without Hp-related gastritis had high negative antibody titers. Endoscopic findings in the stomach or duodenum for patients with high negative antibody titers are shown in **Table 2b**, where 82 (79.6%) had atrophic gastritis.

In UGI examinations, we found that 283 (64.0%) out of 552 patients with gastritis were Hp-positive in the EIA test. Sixty-two (14.0%) of the 442 patients with gastritis had high negative antibody titers and may possibly have had Hp infection. Among 506 subjects without gastritis 34 (6.7%) had a high negative antibody titers.

Factors associated with Hp infection

In the investigation of factors associated with Hp infection, we first performed a single logistic regression analysis. In it, aging, anemia, increase in WBC, decrease

in platelets, hypertension, low HDL-cholesterol, elevated FBS or HbA1c, impaired glucose tolerance, elevated AST and metabolic syndrome were associated with Hp infection ($p < 0.05$) (**Table 3**). In the multiple logistic regression analysis that was then performed, aging, anemia, increased WBC, and elevated AST were associated with Hp infection ($p < 0.05$) (**Table 4**). Since AST has been reported to vary with age or gender[13], we performed the analysis for several age group and gender combinations. In female subjects of 60-64 years old, there was statistically significant association between Hp and AST elevation ($p = 0.044$, odds ratio 1.104 95% CI 1.003–1.216).

Since Hp infection increases with aging, we performed the analysis with the subjects divided into different age groups (**Table 5**). Interestingly, we found that factors associated with Hp infection were different from those identified above. In patients under 40 years old, multiple logistic regression analysis showed that no factors were associated with Hp infection. In the 40–59 years old group, increased WBC and decreased platelet cells ($p = 0.003$, each) were associated with Hp infection. In patients over 60 years old, increased WBC, lower HDL cholesterol levels and smaller abdominal circumference were associated with Hp infection.

Discussion

Recent research has revealed that Hp infection can be present even when the result with a serum anti-antibody EIA test kit is negative[2,3]. It is a problem that there is the possibility of Hp-infected patients with gastritis (atrophic gastritis or nodular gastritis) not receiving proper eradication therapy due to the inability of the EIA kit to detect Hp infection. In addition, accurately diagnosing Hp infection is important for the prevention of gastric cancer, since Hp-related gastritis is a potential precursor of this form of cancer. In this study, 14.2% of patients with Hp-related gastritis had an Hp antibody titer of 3–9 and were therefore considered Hp-negative. These patients accounted for 60–70% of the patients suffering from Hp-related gastritis who were diagnosed as Hp-negative with the EIA kit and this result indicates that a considerable number of patients with Hp-related gastritis were not properly diagnosed regarding Hp infection. These patients had also missed the opportunity to receive eradication therapy to reduce the risk of gastric cancer. Appropriate testing that properly diagnoses Hp infection is necessary for

Table 4. Multiple Logistic Regression Analysis

Factors	*p* value	Odds Ratio	95%CI
Age	<0.001*	1.044	1.035 – 1.054
Anemia	0.045*	1.379	1.022 – 1.810
WBC	<0.001*	1.000	1.000 – 1.000
Plt	0.128	0.999	0.997 – 1.000
HT	0.555	0.932	0.737 – 1.178
HDL cholestterol	0.066	0.995	0.990 – 1.000
Impaired glucose tolerance	0.981	1.003	0.784 – 1.294
AST	0.048*	1.009	1.000 – 1.019
Metabolic syndrome	0.340	0.858	0.626 – 1.175

*Statistically significant. WBC: white blood cell count; Plt: platelet cell count; HT: hypertension; HDL: high density lipoprotein; AST: aspartate aminotransferase; CI: confidence interval

Table 5. Multiple Logistic Regression Analysis

Age group	Factors	*p* value	Odds Ratio	95%CI
Under 40 years old	TC	0.185	0.993	0.983 – 1.003
	TG	0.107	0.995	0.990 – 1.001
	Alcohol consumption	0.060	1.001	1.000 – 1.003
40–59 years old	WBC	0.003*	1.000	1.000 – 1.000
	Plts	0.003*	0.996	0.994 – 0.999
Over 60 years old	WBC	0.014*	1.000	1.000 – 1.000
	Plts	0.319	1.002	0.998 – 1.005
	HDL cholesterol	0.002*	0.984	0.974 – 0.994
	AST	0.061	1.022	0.999 – 1.045
	γ -GTP	0.225	1.003	0.998 – 1.007
	AC	<0.001*	0.958	0.937 – 0.979

*Statistically significant. WBC: white blood cell count; Plts: platelet cells; TC: total cholesterol; TG: triglyceride; HDL: high density lipoprotein; AST: aspartic aminotransferase; γ -GTP: γ -glutamyl transpeptidase; AC: Abdominal circumference; CI: confidence interval

preventing gastric carcinogenesis.

Disorders related to Hp infection have been under investigation for at least a decade[4–7]. We investigated the factors related to Hp infection using data from Ningen Dock, and found that age, anemia, increased WBC, and elevated AST were related to Hp infection. It seemed reasonable that age was aassociated with Hp infection, since Hp infection is already known to increase with aging[14,15]. It has been reported that birth cohort effects reflected a decrease in the rate of Hp infection in successive generations of children as sanitation and living standards improved[15]. Anemia was also observed to be a factor associated with Hp infection in previous studies[4]. Increased WBC was likely associated with Hp infection because bacterial infections usually increase WBC counts. However, the observation that AST is a factor associated with Hp infection is a new finding. Since other liver function tests, such as ALT and γ-GTP, were not found to be associated with Hp infection, this seems to indicate that there may not be a simple relationship between Hp infection and liver damage. AST levels are known to increase with myocardial damage, skeletal muscle damage, or hemolytic anemia. Several studies have reported that Hp infection plays a role in the pathology of coronary artery diseases such as myocardial infarction[16] but few studies have found associations between Hp infection and hemolytic anemia. We suggest that auto-immune disorders play a role in the development of hemolytic anemia after Hp infection, because idiopathic thrombocytopenic purpura (ITP) is also known to be caused by Hp infection[7]. The above studies suggest the possibility that AST is increased by Hp infection due to muscle damage or a kind of hemolytic anemia[17]. Further studies will be needed to clarify the effects of Hp infection on AST.

In conclusion, we found that aging, anemia, increased WBC, and elevated AST were factors associated with Hp infection. Further studies are needed to clarify the role of these factors in the pathophysiology of the disease. Additionally, we found that a considerable number of Hp-infected patients had negative EIA Hp test results. Further improvements in the methods of diagnosing Hp infection will hopefully resolve these problems and improve prospects in gastric cancer prevention.

Conflict of Interest Statement

All authors declare no conflict of interest.

Acknowledgments

We greatly thank the staff of the Center for Preventive Medicine, The Jikei University Hospital for their valuable contributions and support.

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(Received June 10, 2018 ; Accepted November 16, 2018)

Original Article

Ningen Dock International 2019 ; 6 : 56–61

Number of Eosinophils and Incidence of Cancer in a Japanese Population : A Single Institution Study

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Abstract

Objective : Allergies might be associated with a low risk of developing malignant diseases. When an allergy is present, it is thought that the immune surveillance mechanism may inhibit carcinogenesis because the immune system is in a state of hyperreactivity. Usually, eosinophils increase in this state. Therefore, we investigated an association between the number of eosinophils and cancer incidence.

Methods: This cross-sectional study included 31,350 Japanese individuals, and was conducted at the Center for Preventive medicine, NTT Medical Center Tokyo, from 2006 to 2011. Associations of the number of eosinophils with cancer were assessed based on cancer-related information from self-reported medical histories in questionnaires. Absolute eosinophil counts were calculated by multiplying the relative count by the total WBC count.

Results: Among all 31,350 subjects, the mean absolute eosinophil count was 145.8/μL, 21% had allergies and 4.9% had cancer. The incidence of cancers was significantly lower in a group with eosinophil counts >350/μL than in those with eosinophil counts ≤350/μL (3.75% vs 4.95%, p=0.03). Multivariate analysis revealed that allergies and eosinophils >350/μL were independent negative factors for cancer (p<0.0001, p=0.012, respectively).

Conclusions: An increased number of eosinophils was significantly associated with low incidence of cancers. In addition, our results suggested that high eosinophil counts may be inversely associated with cancer risk, regardless of whether allergies are present or not.

Keywords eosinophil, cancer, allergy

Epidemiological studies have reported that there may be an association between allergies and low risk of malignancy. However, their findings were inconsistent, mainly because the relationship between allergies and cancer is complex. Also, the types of cancer and definitions of allergy differed among the studies. The hyperactivated immune system in allergies is thought to act against cancer and in this regard, allergic conditions were reported to be associated with a lower risk of pancreatic cancer, glioma, childhood leukemia, and colorectal cancer[1,2] . On the other hand, chronic inflammation due to allergies is considered to contribute to carcinogenesis[1].

People with allergies are characterized by an increased blood eosinophil count. Eosinophils are considered to play a role in the regulation of the immune response and they release a number of cytotoxic molecules depending on the type of stimuli. The granules of eosinophils contain highly cytotoxic preformed proteins. They also have the capacity to produce costimulatory signals to release cytokines, promoting type 2 immunity[3,4]. Eosinophilia is a prominent feature of allergies and has been found to be inversely associated with various cancers[5,6]. Therefore, we hypothesized that subjects with eosinophilia in Japan would have a lower incidence of cancer. The aim of this study was to investigate whether there is any relationship between allergies, especially in terms of eosinophil count, and cancer incidence.

Methods

Study population

This was a cross-sectional study enrolling subjects from among 59,945 individuals who received general health screening tests at the Center for Preventive Medicine of the Nippon Telegraph and Telephone Corporation (NTT) Medical Center Tokyo, from May 2006 to March 2011. After reviewing the medical re-

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ports of all of the subjects, 28,595 were excluded from the present study because they had visited our center more than twice from May 2006 to March 2011. The analysis for the study used the results for people who visited our hospital for the first time. The present study was performed as a part of the general health check-up program conducted at our center. Most of the study subjects were employees of NTT or relatives of NTT employees and the rest of them were people living in the surrounding area.

All examinations were performed at our institution alone, and the results were analysed statistically. Therefore, the data from the examinations were precise and had little variance. The participants completed self-administered qestionnaires regarding their demographic characteristics and medical histories, and those who failed to complete them were interviewed by well-trained staff. The study protocol was approved by the institutional ethics committee.

Absolute number of eosinophils and cancer

The absolute number of eosinophils was calculated from the complete blood count and the percentage of eosinophils in the hemogram of each subject. Subjects were defined as having allergies based on present allergic diseases (bronchial asthma, allergic rhinitis, hay fever, atopic dermatitis, drug or food allergy) or previous medical history of allergies. They were defined as having cancer based on entries concerning present cancers or past history of cancer in the self-administered questionnaire (esophageal cancer, stomach cancer, colon cancer, liver cancer, gallbladder cancer, lung cancer, thyroid cancer, leukemia, malignant lymphoma, kidney cancer, prostate cancer, uterine cancer, ovarian cancer, breast cancer, pharyngeal cancer, laryngeal cancer), or past history of cancer. They were also defined as having cancer it was discovered in a medical examination at the time. Previously reported criteria were used for definitions of other risk factors, such as hypertension, diabetes, and alcohol consumption[7].

Statistical analysis

The data were presented as mean ± standard deviation (SD) or as percentages (%). Differences between groups were assessed using either the unpaired two-tailed Student's *t* test or the chi-square test for continuous and categorial variables, respectively. For analysis to identify independent risks for cancer, variables with $p<0.05$ in **Table 2** were entered into a multivariate logistic regression model. Continuous variables were transformed into binary data, with 1 representing the presence of the assumed risk factor and 0 otherwise for this division to avoid arbitrary influences. For example, older age was defined as 65 years or older and underweight was defined as BMI less than 18.5 based on the WHO classification. The odds ratios (OR) and 95% confidence intervals (CI) for clinically relevant variables with $p<0.05$ in univariate analysis were included in the data for the final multivariate model.

All analyses were carried out with the R software program.

Results

The basic characteristics of the 31,350 study subjects are shown in **Table 1**. The mean age was 49 years, 26% of them were woman, 5% had diabetes, and 25% of them were current smokers. Allergies were recognized in 6,583 subjects (21%)(**Fig. 1 (a)**). **Fig. 1 (b)** shows that past or current cancers were also noted in 1,533 subjects (4.9%) - including gastrointestinal cancer in 608 (1.9%), lung cancer in 158 (0.5%), prostate cancer in 176 (0.6%) and breast cancer in 177 (0.6%). The distribution of blood eosinophil counts is in the form of a skewed curve with a mean value of 145.8/μL (**Fig. 1 (c)**), the mean counts were 224/μL with allergy vs 125/μL without allergy and 5% of subjects had eosinophil counts greater than 350/μL. **Fig. 1 (d)** shows the number of eosinophils for each 10-year age range, where the numbers of eosinophils were higher in younger subjects. Comparing baseline characteristics for those with or without cancers, there were differences regarding risk factors and lifestyle habits (**Table 2**). Subjects with allergies had significantly lower incidences of the following types of

Table 1. Baseline Characteristics of Subjects

	n=31350 (%)
Age, years	49 ± 12
Male, *n*	23093 (74)
Body mass index, kg/m^2	23.1 ± 3.3
Eoshinophil count /μL	145.8 ± 107.3
WBC count /μL	5650 ± 2367
All cancers, *n*	1533 (4.9)
Lung	158 (0.5)
Esophagus, Stomach, Colon	608 (1.9)
Breast	177 (0.6)
Uterus, Ovary	119 (0.4)
Prostate	176 (0.6)
Thyroid	95 (0.3)
Liver, Gallbladder, Pancreas	42 (0.1)
Kidney	81 (0.3)
Hematological malignancy	77 (0.2)
Others	138 (0.4)
Family history of cancer, *n*	16727 (53)
Allergic conditions, *n*	6583(21)
Hypertension, *n*	4442 (14)
Diabetes mellitus, *n*	1444 (5)
Hemoglobin A1c, %	5.3 ± 0.7
Dyslipidemia, *n*	2700 (9)
Current smoker, *n*	7940 (25)
Alcohol, *n*	18175 (58)

Data are presented as number of patients (%) or mean ± SD. WBC, white blood cell

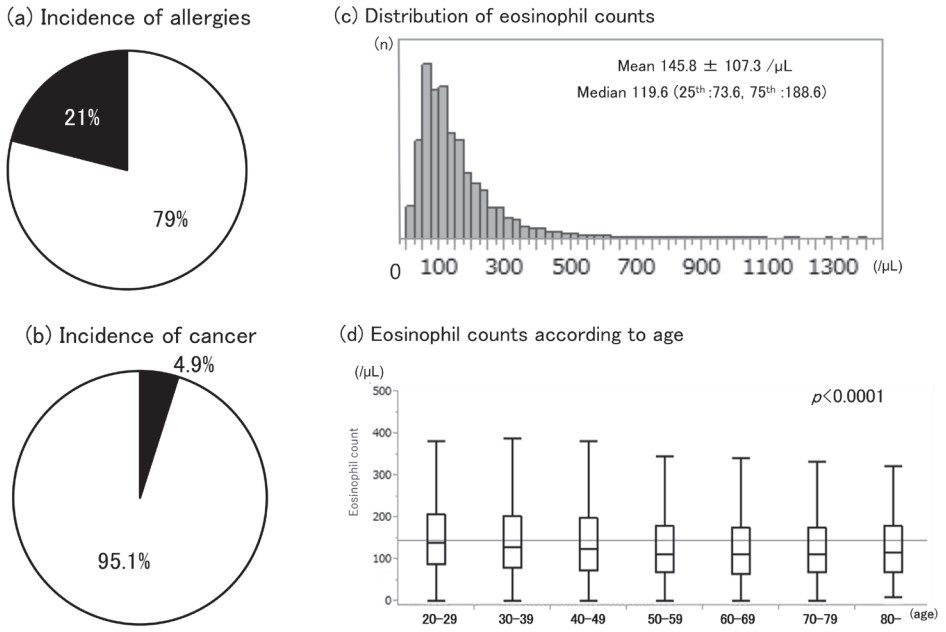

Fig.1. (a) Incidence of Allergies. (b) Incidence of Cancer. (c) Distribution of Eosinophil Counts. (d) Eosinophil Counts for each 10 Year Age Range

Table 2. Baseline Characteristics between Cancer and No-cancer Subjects

	Cancer (+) n = 1533 (%)	Cancer (–) n = 29817 (%)	p-value
Age, years	59 ± 10	48 ± 11	< 0.0001
Male, *n*	1007 (66)	22086 (74)	< 0.0001
Body mass index	22.7 ± 3.3	23.1 ± 3.3	< 0.0001
Eosinophil count /μL	137 ± 105	146 ± 109	< 0.0001
Eosinophil count > 350/μL	57 (4)	1466 (5)	0.03
WBC count /μL	5634 ± 8118	5651 ± 1583	0.79
Family history of cancer, *n*	893 (58)	15834 (53)	< 0.0001
Allergic conditions, *n*	183 (12)	6393 (21)	< 0.0001
Hypertension, *n*	369 (24)	4073 (14)	< 0.0001
Diabetes mellitus, *n*	188 (13)	1326 (4)	< 0.0001
Dyslipidemia, *n*	212 (14)	2488 (8)	< 0.0001
Current smoker, *n*	230 (15)	7710 (26)	< 0.0001
Alcohol, *n*	794 (52)	17381 (58)	< 0.0001

Data are presented as number of patient (%) or mean ± SD. WBC, white blood cell

cancer: lung cancer, gastrointestinal cancer, breast cancer, liver/gallbladder cancers, kidney cancer, and prostate cancer. The incidence of cancers was significantly lower in subjects with eosinophil counts >350/μL compared to those with eosinophil counts ≤350/μL (**Fig.2**). To identify independent risk factors of cancer, significant variables in **Table 2**, such as elderly (≥65 years), male, lower BMI (<18.5), eosinophil count >350/μL, family history of cancer, allergic condition, hypertension, diabetes, dyslipidemia, smoker, and alcohol, were entered into multivariate analysis. There were positive correlations among elderly, lower BMI, female, hypertension, diabetes and family history of cancer. However, allergic condi-

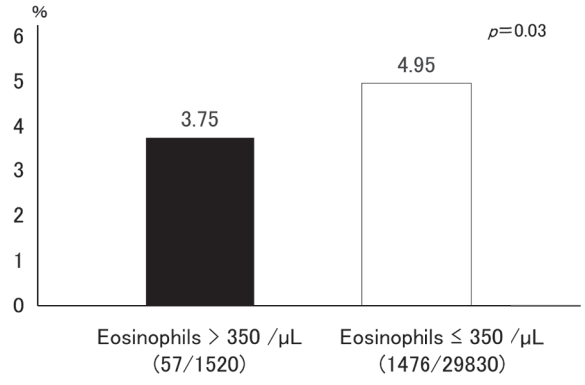

Fig. 2. Incidence of Cancer According to Eosinophil Counts

tions (OR=0.49, 95%CI 0.40–0.59 , $p<0.0001$) and higher blood eosinophil counts >350/μL (OR=0.65, 95%CI 0.48–0.91, $p=0.012$) were independent protective factors for cancer (**Fig. 3**). Among the elderly (≥ 65 years), eosinophil counts were significantly lower in subjects with cancers compared to those without cancers. However, the difference in eosinophil counts between with and without cancer in non-elderly subjects (<65 years) was small (**Fig. 4**).

Discussion

In the present study, we evaluated whether absolute eosinophil counts were associated with the incidence of cancers in subjects who underwent general health screening, in order to test the hypothesis that the incidence of several cancers in subjects with eosinophilia is low. Regardless of whether an allergic condition was present or not, high eosinophil counts were inversely associated with low cancer incidence.

Six factors related to the risk of cancer were consistent with those reported by previous studies[8–10]. It is well known that age is a strong risk factor for cancer. In this regard, the present study showed the number of eosinophils was high in non-elderly subjects(<65 years) and low in elderly subjects (≥65 years). Also, in non-elderly subjects eosinophil counts were not significantly different between those with cancer and those without. The incidence of cancer is low in non-elderly subjects and differences in eosinophil numbers may be small.

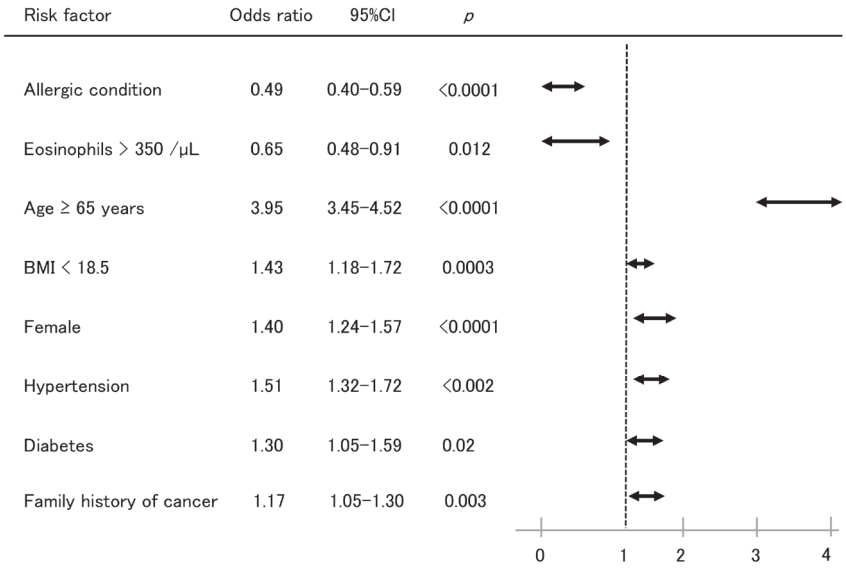

Fig.3. Risk Factors of Cancer by Multivariate Analysis

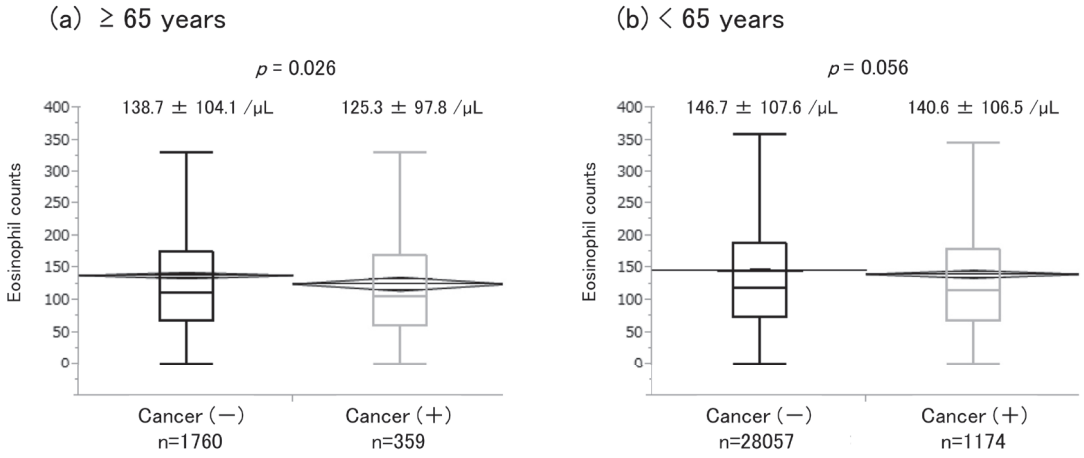

Fig.4. Eosinophil Counts between Subjects with and without Cancer
(a) Elderly subjects (≥ 65 years), (b) Non-elderly subjects (<65 years).

Although several studies have investigated the relationship between allergies and risk of cancers, their findings have been controversial[11–20]. In a case control study with 532 cases, Holly EA *et al*. reported that a history of allergy was associated with a reduced risk of pancreatic cancer[19], and reduced pancreatic cancer risk was observed consistently with an increased number of allergies. Also, in a study conducted on 252 cases of childhood acute lymphocytic leukemia (ALL), self-reported allergic history and present allergies (respiratory, food, other clinical allergy) were strongly and inversely associated with ALL[21]. However, some studies noted increased cancer risk with allergies[22], and a few found no association between them and cancer risk[23].

To our knowledge, the present cross-sectional study is the first in Japan to find that the number of eosinophils was associated with low incidence of some cancers for cross sectional study. The frequency of cancer in the subjects with allergies was approximately half that in those without allergies. Although a precise reason for the association between allergic conditions and a reduced risk of cancer remains unclear, it may be explained by enhanced immune-surveillance, which helps to detect and eradicate pre-malignant cells and thus reduce cancer risk. Another hypothesis proposed involves prophylaxis, suggesting that the physical effects of allergic reactions in some tissues may clear mutagenic triggers before malignant transformations can occur.

Other findings suggest that eosinophils may exert an inhibitory effect against the development of cancers. In patients with allergies, eosinophils usually increase in peripheral blood and tissues, and allergy severity has been reported to be correlated with the peripheral blood eosinophil count, and IgE[24].

Eosinophils produce and release many cytokines, preferentially those that mainly promote type 2 immunity and they may participate in immune surveillance by acting synergistically with macrophages and releasing immunoregulatory cytokines responsible for antitumor responses[25]. In addition, *in vitro* and *in vivo* studies have shown that eosinophils may produce granule proteins that are highly tumor cytotoxic[26]. Importantly, eosinophils from allergic donors were more cytotoxic than those from non-allergic donors[27], which suggests that the allergic state favours antitumor processes.

An interesting finding of the present study was that high eosinophil counts (cut-off point > 350) were protective in antitumor processes even in those who had no obvious allergic condition. Also, Taghizadeh N *et al*. reported that high eosinophil counts were associated with a reduced risk of colorectal cancer mortality among ever smokers and males[28]. In addition, human eosinophils have been reported to induce colon cancer cell death *in vitro*, implying mechanisms involving innate receptors (TCRγδ/CD3 complex, TLR2) and mediators such as alpha-defensins, TNF-α, granzyme A and IL-18[27,29,30]. Furthermore, tumoricidal functions of eosinophils may be target antigen-specific and differ among individuals, and tumor antigen-specific IgE has been shown to trigger eosinophil-mediated tumor cell death by cytotoxic mechanisms[31]. Therefore, high levels of eosinophils may play a protective role against cancer development in healthy subjects.

Before drawing conclusions, the findings of the present study should be carefully interpreted. Firstly, it was a cross-sectional observational study based on data from a single institution and therefore may have limited applicability to all patients in Japan with cancers. Secondly, the effect of selection bias should be considered. Approximately 70% of our subjects were healthy male office workers who ranged in age from 40 to 60 years. Another reason that they do not accurately represent the whole Japanese population is that they included subjects not connected with NTT who were assumed to be highly health conscious individuals because they had had decided to undergo health check-ups by themselves. As a final limitation, allergic condition, medical histories and lifestyle habits were evaluated using a self-administered questionnaire.

In conclusion, an increased number of eosinophils suggesting an allergic condition was associated with low incidence of cancers in our subjects. In particular, we found that high levels of eosinophils were independently associated with a low incidence of cancer. The relationship between allergies and the risk of cancer should be further examined in future longitudinal studies.

Conflicts of Interest

All authors report that they have no disclosures to make relating to the publication of this study.

Acknowledgement

We thank Dr. Akio Urabe for reviewing this manuscript.

An abstract of this article was presented at the 54th Scientific Meeting of the Japan Society of Ningen Dock in Hamamatsu in 2013.

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(Received July 20, 2018 ; Accepted January 23, 2019)

Original Article

Ningen Dock International 2019 ; 6 : 62–68

Epidemiological Features of and Screening for Polycystic Kidney Disease in Japan

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Abstract

Objective: Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited renal disease. Approximately 50% of the affected patients suffer end-stage renal failure, requiring dialysis. In recent years, ADPKD has gained substantial attention due to advances in its treatment and its coverage by the financial aid system for treatment of intractable diseases in Japan. In this study, we evaluated the current status of patients with ADPKD undergoing health check-up at our facilities and examined the validity of the screening method.

Methods: In total, 47,383 individuals who underwent health check-up at our facilities between April 2017 and March 2018 were screened using abdominal ultrasonography focusing on the number of renal cysts. Individuals in whom ADPKD was suspected were interviewed further and advised to undergo close examination, the results of which were analyzed.

Results: Among the study subjects, the prevalence rate of ADPKD was 68–137 per 100,000 population, and the positive predictive value of our screening method was 36.2%. Among the newly diagnosed ADPKD patients, only 11.8% had renal ultrasonographic findings that required close examination according to the current Abdominal Ultrasonographic Screening Manual, which does not include number of renal cysts as a standard criterion.

Conclusions: The prevalence rate of ADPKD was considerably higher than that previously reported in Japan (≈25 per 100,000 population), suggesting a high number of undiagnosed cases. ADPKD may be detected and treated at early stages by screening based on the number of renal cysts.

Keywords polycystic kidney disease, dialysis, health check-up, Abdominal Ultrasonographic Screening Manual

Globally, autosomal dominant polycystic kidney disease (ADPKD) is considered to be the most common inherited renal disease. In affected adults, multiple cysts develop and grow progressively in both kidneys, resulting in disease progression to renal failure. In fact, approximately half of ADPKD patients suffer end-stage renal failure by 60 years of age and require dialysis[1–3]. ADPKD, which ranks fourth among the indications for dialysis[4,5], is a condition that cannot be disregarded not only from the perspective of the quality of life of patients but also that of health economics. Also, fatal complications, such as cyst infections and cerebral aneurysms, may occur even prior to the development of renal failure[6,7]. Compared to ruptured cerebral aneurysms associated with other diseases, those due to ADPKD reportedly occur at a 5-fold higher frequency and at an age at least 10 years younger[3].

Nevertheless, to date, efforts in the early diagnosis of ADPKD have been limited because no effective treatment existed until recently and patients are mostly asymptomatic until they reach their 40s. However, vasopressin receptor antagonists, which became available in 2014, have enabled the increased renal volume and decreased renal function to be effectively suppressed[8]. In 2015, the new Intractable Disease Health Care Act came into effect in Japan, and ADPKD was covered by the system of financial aid for the treatment of intractable diseases, providing greater support for affected patients[9]. In consideration of these changes in the management of ADPKD, we surveyed subjects undergoing health check-up at our facilities to determine the current status of ADPKD, and evaluated the epidemiological features and disease screening method.

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Methods

We studied a total of 47,383 individuals who underwent hospitalized comprehensive or ambulatory health check-up at our facilities between April 2017 and March 2018 (**Table 1**, **Fig. 1**).

According to the Guideline Recommendations for the Diagnosis and Management of Autosomal Dominant Polycystic Kidney Disease (2nd edition), ADPKD is diagnosed based on familial incidence as well as computed tomography, magnetic resonance imaging (MRI), and abdominal ultrasonography findings (**Fig. 2**)[10]. However, in this study, to examine whether patients with ADPKD can be accurately identified in a standard health check-up, ≥3 cysts in each kidney on abdominal ultrasonography was taken to indicate a fair suspicion of ADPKD, whereas ≥5 cysts in each kidney was taken to indicate a strong suspicion (**Fig. 3**). Ultrasonographic examinations were performed using ProSound α7 (Hitachi Ltd., Tokyo, Japan) fitted with a Hitachi UST-9130 probe, 2–6 MHz.

The subjects who met these criteria had additional interviews at our facilities and were advised to undergo further examination at hospitals, where the disease was precisely diagnosed according to the guidelines (**Fig. 2**).

Table 1. Composition of Subjects who Underwent Health Check-up

Subjects	Number of subjects	Mean age (years)	Age ranges (years)
Overall	47383	49.7	17–90
Men	30562	50.5	17–88
Women	16821	48.2	20–90

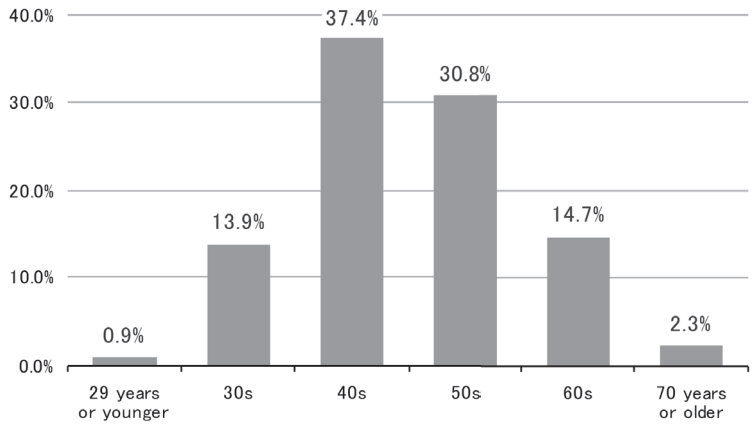

Fig.1. Age Composition of Subjects who Underwent Health Check-up in This Study

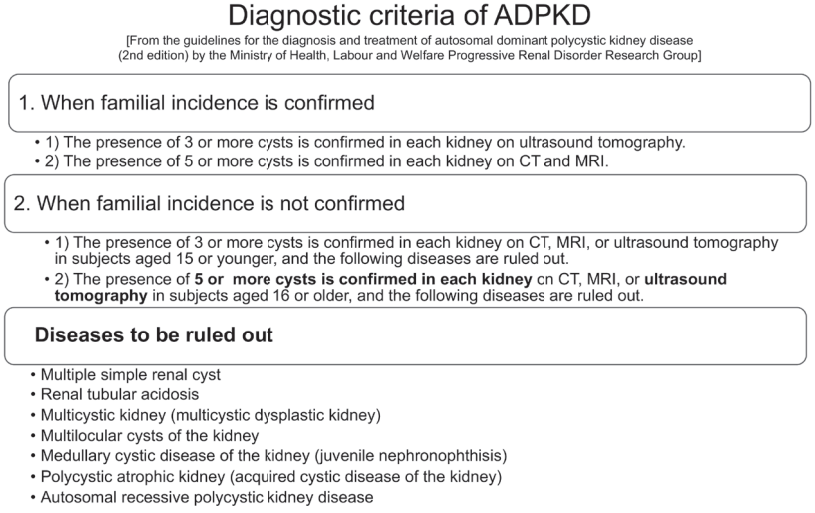

Fig.2. Diagnostic Criteria of Autosomal Dominant Polycystic Kidney Disease

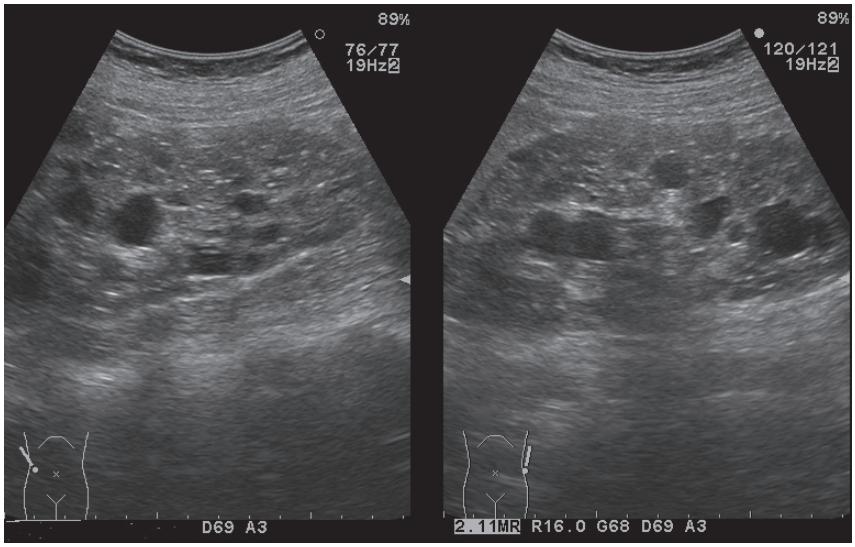

Fig.3. Representative Findings for Bilateral Multiple Renal Cysts on Abdominal Ultrasonography

Table 2. Positive Predictive Value (PPV) and Estimated Prevalence Rates of Autosomal Dominant Polycystic Kidney Disease (ADPKD) Based on Our Survey Results (47,383 Health Check-up Subjects)

Renal Cyst Findings	No. of Cases	ADPKD (Already Diagnosed) (A)	Further Examination Advised (B)	Successfully Followed Up (C)	ADPKD (Newly Diagnosed) (D)	PPV (E) = D/C	ADPKD (Estimated) (B × E) + A
≥ 5 cysts in both kidneys	97	15	82	28	14	50.0%	56
≥ 3 cysts in both kidneys and <5 in at least one kidney	55	0	55	19	3	15.8%	9
Total	152	15	137	47	17	36.2%	65
ADPKD confirmed or estimated				*Confirmed*	32(15 + 17)	*Estimated*	65
Prevalence rate					0.07%		0.14%
Prevalence rate per 100,000 population					68		137

The results of interviews and data from additional examinations were used to determine the actual prevalence rate of ADPKD, positive predictive value (PPV) of ultrasonographic screening, and characteristic features of the medical history of patients with ADPKD.

All procedures were performed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. The Ethics Committee of Clinical Trials of our hospital approved the study, and informed consent was obtained from all examinees prior to their inclusion in the study.

Results

Of 97 (0.20%) subjects who were strongly suspected to have ADPKD based on abdominal ultrasonography findings of ≥5 cysts in both kidneys, 15 had previously been diagnosed with ADPKD. The remaining 82 subjects were advised to undergo further examination, and 14 of 28 who could be followed up were newly diagnosed with ADPKD. Thus, the PPV was found to be 50.0% (14/28). Applying the PPV to all subjects including those who could not be followed up, we estimated that 56 of the 97 strongly suspected subjects had ADPKD (**Table 2**).

Fifty-five (0.12%) subjects who had ≥3 cysts in both kidneys but <5 cysts in at least one kidney were fairly suspected to have ADPKD. Among them, 3 of 19 subjects who could be followed up were diagnosed with ADPKD (PPV 15.8%), suggesting that 9 of the 55 fairly suspected subjects had ADPKD, when subjects who could not be followed up were included.

Based on these results and the size of the population in this survey, the prevalence rate obtained for the patients confirmed to have ADPKD was 68 per 100,000 population (0.07%). When the subjects who were not followed up were included, using the PPV, the estimated prevalence rate increased to 137 per 100,000 population (0.14%).

Table 3 shows the age and sex composition of the 32 subjects confirmed to have ADPKD. The mean age of all patients was 50.9 years (range, 34–64 years). Regarding the male-to-female ratio, male patients in this

Table 3. Composition of 32 Patients Confirmed to have Autosomal Dominant Polycystic Kidney Disease (ADPKD)

	Total No. patients	Mean Age (Yrs.)	30s	40s	50s	60s
All patients with ADPKD	32 (17*)	50.9 (34–64)	7 (3*)	6 (4*)	11 (5*)	8 (5*)
Men	22 (14*)	53.0 (38–64)	4 (2*)	3 (2*)	8 (5*)	7 (5*)
Women	10 (3*)	46.2 (34–60)	3 (1*)	3 (2*)	3 (0*)	1 (0*)

*Number of patients newly diagnosed with ADPKD

Table 4. Medical Histories from Health Check-up of 32 Subjects Confirmed to have Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Medical History of Subjects	No. of Subjects/Valid response	Frequency	National Epidemiological Survey[11]
Hepatic cyst	28/32	87.5%	60%–80%
Hypertension	18/28	64.3%	60%–80%
Renal calculus	4/28	14.3%	10%–20%
Colonic diverticula	1/28	3.6%	80%
Cerebral hemorrhage	1/28	3.6%	8%–10%
Cerebral aneurysm	0/28	0%	5%–12%
Mitral regurgitation	0/28	0%	20%–25%

Table 5. Family Histories of 32 Subjects who Underwent Health Check-up and were Confirmed to have Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Family History (up to Third-Degree)	No. of Subjects/Valid response	Frequency
Polycystic kidney disease	16/27	59.3%
Chronic renal failure	7/27	25.9%
Dialysis	13/27	48.1%
Renal transplant	0/27	0%
Cerebral hemorrhage	9/27	33.3%

group accounted for a slightly greater proportion than in the overall study population (**Table 1**). Analysis by age group showed that subjects aged ≥50 years accounted for 59.4% (19/32) of the study population with ADPKD, and this trend was also observed in the newly diagnosed patients.

The 32 patients with ADPKD were additionally interviewed regarding their medical histories. Consistent with a previous epidemiological study[11], presence of a hepatic cyst on abdominal ultrasonography or a history of hypertension was noted in >80% and >60% of the present patients, respectively (**Table 4**). On the other hand, cerebral hemorrhage, mitral valve disease, and colonic diverticula, which are considered common during the course of this disease, were either absent or noted only in a few patients in the present study.

Polycystic kidney disease was noted in approximately 60% of the family histories of 27 of the 32 patients with ADPKD, as familial incidence is specified in the diagnostic criteria for ADPKD (**Table 5**)[10]. Remarkably, approximately half of the patients had family members on dialysis, and one-third had family members who had suffered cerebral hemorrhage.

Currently, in Japan, the most commonly used reference guide for abdominal ultrasonographic diagnosis during health screening is the Abdominal Ultrasonographic Screening Manual[12] issued by the Japan Society of Ningen Dock, Japanese Society of Gastrointestinal Cancer Screening, and Japan Society of Ultrasonics in Medicine. The manual generally recommends follow up for the finding of multiple cysts in both kidneys (class C), and 2 of the 17 study patients (11.8%) with newly diagnosed ADPKD were recommended to undergo close examination due to the ultrasonographic finding of bilateral renal enlargement of ≥12 cm (class D; **Table 6-1**). Based on the serum creatinine level criteria provided by the Japan Society of Ningen Dock, another patient was determined to be class D (**Table 6-2**). Thus, in total, only 3 of the 17 patients (17.6%) were recommended to undergo close examination due to meeting overall criteria in their health check-up.

Eleven of the 17 newly diagnosed patients had also undergone a health check-up at our facilities in 2016 so they could be followed up for health changes that occurred over a year based on ultrasonography. The average rate of increase in the long diameter of the kidney was 4.3–5.6% (**Table 7**).

Table 6-1. Findings Based on Abdominal Ultrasonographic Screening Manual for 17 Subjects Newly Diagnosed with Autosomal Dominant Polycystic Kidney Disease (ADPKD) in This Study

Findings on Ultrasound Imaging	Category	Ultrasound Finding	Assessment Class	No. of Patients
Cystic lesion: Cysts of varying size aggregated bilaterally and making renal parenchyma obscure	3	Suspected polycystic kidney disease	C	17
Calcification:	2	Nephrocalcinosis or Nephrolithiasis	B	10
Abnormal morphology: maximum diameter is ≥ 12 cm on both sides	3	Renal enlargement	D2	2

Because some patients had multiple findings, the total number of cases with findings did not tally with the overall number of patients.
Assessment classes A: No abnormality, B: Mild abnormality, C: Follow-up, re-examination and lifestyle guidance required, and D: Medical attention required (D1: Medical treatment required and D2: Close examination required)

Table 6-2. Serum Creatinine Levels Based on Criteria of Japan Society of Ningen Dock for 17 Subjects who were Newly Diagnosed with Autosomal Dominant Polycystic Kidney Disease (ADPKD) in This Study

	A	B	C	D
Criteria	M : ≤ 1.00 F : ≤ 0.70	M : 1.01–1.09 F : 0.71–0.79	M : 1.10–1.29 F : 0.80–0.99	M : ≥ 1.30 F : ≥ 1.00
Number of patients	12	2	2	1

Assessment classes A: No abnormality, B: Mild abnormality, C: Follow-up, re-examination and lifestyle guidance required, and D: Medical attention required (D1: Medical treatment required and D2: Close examination required)

Table 7. Rate of Increase in Renal Long Diameter from Ultrasonography and Serum Creatinine Level for 11 Newly Diagnosed Patients who also Underwent a Health Check-up in 2016 at Our Facilities

No	Age/Sex	2017/2016 Lt. renal Long diameter (cm)	Increase rate (%)	2017/2016 Rt. renal Long diameter (cm)	Increase rate (%)	2017/2016 Serum creatinine (mg/dL)
1	38/M	10.5/10.5	0%	9.9/9.6	3.7%	0.87/0.83
2	41/M	*14.6/*14.4	1.4%	*16.1/*15.8	0.9%	1.02/0.98
3	43/F	*13.0/10.6	22.6%	*14.2/11.7	10.3%	0.59/0.55
4	48/M	11.9/11.5	3.5%	11.9/11.9	0%	1.22/1.11
5	55/M	10.1/9.7	4.1%	11.0/9.7	7.6%	0.93/0.88
6	57/M	8.0/8.0	0%	8.0/8.0	0%	*1.39/*1.32
7	59/M	10.4/10.7	-2.8%	11.3/11.1	0.6%	0.72/0.66
8	59/M	8.5/8.3	2.4%	8.5/6.8	19.1%	0.99/0.80
9	61/M	10.2/9.0	13.3%	9.6/9.2	4.1%	0.72/0.66
10	62/M	9.0/8.8	2.3%	10.2/8.7	14.3%	0.79/0.76
11	64/M	10.4/10.4	0%	13.4/13.0	1.4%	0.98/0.96
Average	53	10.6/10.2	4.3%	11.3/10.5	5.6%	

*Recommended to undergo close examination due to criteria of current medical check-up

Discussion

In Japan, the number of patients requiring dialysis to compensate for reduced renal function has been increasing due to the rapidly aging population. At the end of 2016, there were approximately 330,000 patients on dialysis, an increase of approximately 60% on the corresponding number at the end of 2000[13], and the annual medical expenditure for this had risen to 1.6 trillion yen[14], which accounts for approximately 4% of total medical expenditure (approximately 40 trillion yen) in Japan. In this situation, since polycystic kidney disease is a highly ranked indication for dialysis[4], its early detection to address deterioration of renal function is of critical importance not only from the perspective of maintaining the quality of life of patients but also from that of health economics.

A previous Japanese epidemiological study reported the prevalence rate of ADPKD in the Japanese population to be approximately 24.8 per 100,000 population, with the highest rate of 26.1 per 100,000 population in those aged 55–59 years[2]. The prevalence rates in our study were approximately 2.6- to 5.5-fold higher. They were also higher than those in an overseas study[15], and very close to the rates estimated from population sequencing[16] and autopsy results from various hospitals (**Table 8**)[1].

However, based on the current Abdominal Ultrasonographic Screening Manual, only 2 of 17 new patients

Table 8. Comparison of Autosomal Dominant Polycystic Kidney Disease (ADPKD) Prevalence Rates Obtained in Present Study and Those of Previous Japanese and Overseas Studies

	Present Study	Japanese Epidemiological Study[2]	Overseas Study[15]	Population Sequencing Study[16]	Estimation from Autopsy Data[1]
Prevalence rate	1 per 730–1471	1 per 4033	1 per 3019	1 per 1075	1 per 350–780
Per 100,000 population	68–137	24.8	33.1	93	128–286

with ADPKD were aggressively determined to be class D cases (close examination required). If potential patients are determined to be Class C (follow-up required) for polycystic kidney disease, this is unlikely to motivate them to undergo further examination because most are asymptomatic until renal failure advances. Therefore, our results suggest that a large number of people remain untreated despite the results of abdominal ultrasonography performed during health check-up.

Optimally, examinations should be faithfully implemented according to the guidelines for the diagnosis of ADPKD so that examinees can be screened for this disease with high sensitivity and specificity (**Fig. 2**)[10]. However, it is not practical to use operational rules biased toward the detection of a particular disease in general health check-up that aim to screen for a wide variety of diseases. However, as our screening method simply focuses on the number of cysts in both kidneys, it can be applied during the usual health check-up program. The PPV was 36.2% in cases with ≥3 cysts and 50.0% in cases with ≥5 cysts in both kidneys (**Table 2**) so we believe that these screening criteria are sufficiently practicable.

The rate of increase in renal volume in ADPKD patients has been found to be approximately 5.5% per year[8]. Also, while sonographic measurement of kidney volume is inaccurate, the long diameter of the kidney was seen to be a reproducible measurement, and its correlation with MRI volume was 0.84[17]. In our sonographic study, the average rate of increase in the long diameter of the kidney based on ultrasonography was 4.3–5.6% in newly diagnosed patients, suggesting that it is an effective indicator if past findings can be compared during health check-up.

If detailed information can be obtained from individual subjects regarding ADPKD in additional interviews while explaining the check-up results, as during hospitalized comprehensive health check-up, it is possible to further increase the accuracy of screening. A medical history of hypertension or hepatic cysts and/or family history of ADPKD, cerebral hemorrhage, or dialysis may suggest the need for actively carrying out close examination even when the number of cysts is <3 in at least one kidney. On the other hand, in our study, medical histories of cerebral aneurysm, mitral valve disease, and colonic diverticula were determined in a few subjects with ADPKD. This was presumably because these subjects, being asymptomatic, had no reason to undergo head magnetic resonance angiography, echocardiography, or colonoscopy. The use of these conditions in medical history as criteria for screening is inappropriate because they often remain undetected.

Conclusion

The prevalence rate of ADPKD obtained in this study was approximately 2.6- to 5.5-fold higher than the corresponding rate previously reported in Japan. It is expected that improved criteria based on the number of renal cysts in abdominal ultrasonographic examination combined with rate of increase in the long diameter of the kidney and medical or family history will facilitate effective classification of patients with ADPKD. Early detection using abdominal ultrasonography during health check-up and initiation of treatment will prevent affected patients from suffering deterioration of renal function and requiring dialysis. This will substantially contribute to improving their quality of life as well as to reducing medical expenditure in Japan.

Conflict of Interest

The authors declare that they have no conflict of interest.

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(Received November 6, 2018 ; Accepted January 29, 2019)

Acknowledgments

We are very grateful to the following individuals who served as reviewers for the papers submitted to Ningen Dock International Vol. 6 No. 1, March 2019.
I sincerely thank their kind cooperation.

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The Regulations of the International Society of Ningen Dock

Article 1
Name
The name of the association shall be the International Society of Ningen Dock.

Article 2
Office
The Society has its principal office in Japan Society of Ningen Dock.

Article 3
Aims
The Society, an organization of Japan Society of Ningen Dock for international operations, aims to contribute to global health promotion by enhancing the development of ningen dock as a medical check-up system.

Article 4
Tasks
The Society conducts the following tasks to achieve the aims described in the preceding section.
1. Holds congress (World Congress on Ningen Dock), board meetings, lectures, and committee meetings
2. Publishes journals and news magazines
3. Communicates and cooperates with related academic societies both in Japan and overseas
4. Promotes research activities in ningen dock and related fields
5. Does whatever is necessary to achieve the aims of the Society

Article 5
Membership
1. The Society consists of the following members
   1) Regular member
      A regular member shall be a member of the International Society of Ningen Dock who agrees to the aims of the Society, and has expert knowledge, techniques, or experience in the areas associated with the Society.
   2) Supporting member
      A supporting member shall be a person, a corporation, or a group that agrees to the aims of the Society, and supports its programs.
   3) Honorary member
      An honorary member shall be recommended, from those who have significantly contributed to the areas associated with the Society, by the executive board.
2. Those who want to apply for regular or supporting membership of the Society shall submit the prescribed application form with the membership fee.
3. The board meeting will process applications mentioned in the preceding section, and promptly notify the applicants of its decision.

Article 6
Officials
1. The Society shall appoint the following honorary advisors and officials.

Honorary advisor: Number not decided
Congress president: 1
President: 1
Vice president: 3 (from Japan : 2, overseas: 1)
Board members: up to 25 (from Japan : 15 or less, overseas : 10 or less)
Auditor: 2

Article 7
Honorary advisor
1. An honorary advisor shall be appointed by the president from those who have contributed to the development of the Society for a long period, and approved by the executive board.
2. Honorary advisors shall be eligible to attend the board meeting, and to express opinions; honorary advisors will not have voting rights.

Article 8
Congress president
1. The congress president shall be recommended by the executive board and appointed by the president.
2. The congress president shall represent the Society and host the World Congress on Ningen Dock as a scientific meeting.

Article 9
President
1. The president shall be selected by and from among board members and delegated by the president of Japan Society of Ningen Dock.
2. The president shall preside the Society.

Article 10
Vice president
1. The vice president shall be appointed, from among board members, by the president.
2. The vice president shall assist the president. In the case of accident, one of the vice presidents will be appointed by the president and will temporarily take over the duties.

Article 11
Board members
1. Board members from Japan shall be selected among candidates from regular members at Japan Society of Ningen Dock.
2. Overseas board members shall be selected at the recommendation of the executive board.
3. Board members execute duties for the Society under the orders from the president.
4. Board members, together with the president and the vice president, comprise the executive board.

Article 12
Board meeting
1. The president will call a board meeting on an as-needed basis, and serves as the chairman of the meeting.
2. The board meeting will pass resolutions on important matters of the Society.
3. The board meeting shall have the right to start proceedings if the majority of all the board members (including a letter of proxy) attend the meeting.
4. The board meeting shall pass resolutions with the majority votes of attendances.

Article 13
Auditor
Auditors shall audit accounts of the Society, and report to the board meeting.

Article 14
Commissioner
For the aims of successful programs of the Society, the president will set up committees and divisions through the resolutions of the executive board, and delegate the commissioners to regular members or other members of the Society.

Article 15
Accounting
1. The fiscal year for the Society starts on April 1 every year and ends on March 31 the following year.
2. Expenses required for the Society shall be covered by the following revenues.
   1) Membership fees
   2) Grants
   3) Donations
   4) Others

Article 16
Modification of rules
The rules of the Society can be amended by the resolution of the executive board.

Article 17
Miscellaneous provisions
Detailed regulations necessary for the enforcement of the rules of the Society are defined elsewhere by the president with the approval of the executive board.

Article 18
Additional clause
The Regulations of the International Society of Ningen Dock will come into effect on September 15, 2006.

Detailed Regulations of the International Society of Ningen Dock

Detailed regulations of the International Society of Ningen Dock are defined as follows:

(Detailed regulations on members)

Article 1

1. Members shall pay the following annual membership fee; honorary members will be exempt from membership fee.
   1) Regular member : 2,000 yen
   2) Supporting member : from one unit (unit: 20,000 yen)
2. Annual membership fee paid shall not be refunded for any reason.
3. Members with foreign citizenship shall pay a 3-year membership fee of 50 dollars.

Article 2

Members will be given priority in the following events :

1) Participation in scientific meetings hosted by the Society;
2) Contributions of articles to and receipt of the journal of the Society.

Article 3

Members shall lose their memberships in the event of the following:

1) Withdrawal from membership;
2) Adjudication of incompetence or quasi-incompetence;
3) Death or adjudication of disappearance, or dissolution of the group in the case of a member of a supporting group;
4) Delinquency in payment of membership fee for over three year.

Article 4

Those intending to withdraw from the Society must submit the notice of withdrawal in the prescribed form to be approved by the executive board.

Article 5

The Society can expel a member to whom either of the following would apply, with a resolution of the executive board:

1) Those who violate their duty as members of the Society;
2) Those who damage the honor of members of the Society or act against the aims of the Society.

Article 6

Those who satisfy Sections 1 and 2 of Article 5 of the Regulations of the International Society of Ningen Dock will be accepted as members of the Society.

(Detailed regulations on officials)

Article 7

1. The president will be selected from the board members of Japan Society of Ningen Dock.
2. In principle, the majority of board members from Japan will be selected from among the board members of Japan Society of Ningen Dock.

3. Overseas board members will essentially be selected from Asia, Pacific Rim, North America, or Europe.

Article 8

1. The term of the congress president will be from the end of the congress of which he/she is in charge to the next congress.
2. The term of board members will be six years (two terms of three years).

(Detailed regulations on congress and board meeting)

Article 9

Congress and board meeting will be held as follows :

1) The title of the congress will be World Congress on Ningen Dock.
2) In principle, the congress and the board meeting will be held once every three years; with the resolution of the executive board, however, the congress and the board meeting will be held as needed.
3) The congress and the board meeting will be held at the same time.
4) The name of the congress president and the location of the next congress will be announced.

Article 10

1. Those who want to take part in the congress shall pay the participation fee, which is defined separately.
2. Participation fee for the congress will be defined accordingly by the congress president.
3. Only regular members shall be allowed to present the results of their studies, except those who have been approved by the congress president.

(Enforcement of the detailed regulations)

Article 11

1. The detailed regulations will come into effect on September 15, 2006.

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Ningen Dock International is the official journal of Japan Society of Ningen Dock, in which original articles, case reports, and review articles in both Japanese and English are published. Ningen Dock accepts only manuscripts that are original work in the field of ningen dock and related areas not previously published or being considered for publication elsewhere, except as abstracts. The manuscripts published in Ningen Dock will appear on the website of our society.

If the manuscript concerns a clinical study, it must be in accordance with the Declaration of Helsinki of 1964 (subsequent revisions included). Therefore, for a manuscript whose content is epidemiological or clinical research, the approval of the facility's Institutional Review Board (IRB) or the Ethics Committee of Japanese Society of Ningen Dock must have been obtained for the study described. Also, in the text, it should be indicated that informed consent has been obtained from subjects. Additionally, for case reports, it should be stated that adequate care has been taken to ensure the privacy of the subject concerned.

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References should be numbered consecutively in order of appearance in the text and cited in the text using superscript numbers. For example, according to the study by Sasamori[1]. For journals, the names and initials of the first three authors, followed by "*et al.*" if there are other coauthors, the complete title, abbreviated journal name according to Index Medicus, volume, beginning and end pages, and year should be included. For books, the names and initials of the first three authors, followed by "et al." if there are other coauthors, the complete title, book name, edition number, beginning and end pages, name and city of publisher, and year should be included. Examples of references are given below.

Journal: Ishizaka N, Ishizaka Y, Nagai R, *et al.*: Association between white cell count and carotid arteriosclerosis in Japanese smokers. Atherosclerosis 2004; 175: 95-100.

Book: Kaplan NM: Measurement of blood pressure. In: Kaplan NM(ed), Kaplan's Clinical Hypertension. 7th ed., Lippincott William & Wilkins, Philadelphia, 2002, 25-55.

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Tables should be cited in the text, and numbered sequentially with Arabic numerals. Each table should be given a number and a brief informative title, and should appear on a separate page. Explain in footnotes all abbreviations used.

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Figures should be cited in the text, and numbered sequentially with Arabic numerals. A brief descriptive legend should be provided for each figure. Legends are part of the text, and should be appended to it on a separate page. Color figures can be reproduces if necessary, but the authors will be expected to contribute towards the cost of publication.

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Abbreviations

1	**1,5-AG**	1,5-anhydroglucitol	61	**hCG**	human chorionic gonadotropin
2	**17-OHCS**	17α-hydroxycorticosteroid	62	**HCV**	hepatitis C virus
3	**95% CI**	95% confidence interval	63	**HDL-C**	high-density lipoprotein cholesterol
4	**α-GI**	α-glucosidase inhibitor	64	**HLA**	histocompatibility [leucocyte] antigen
5	**β_2-MG**	β_2-microglobulin	65	**HPLC**	high-performance liquid chromatography
6	**γ-GTP**	γ-glutamyl transpeptidase	66	**Ht**	hematocrit
7	**A/G ratio**	albumin-globulin ratio	67	**ICD**	International Classification of Disease
8	**ABI**	ankle-brachial index	68	**ICU**	intensive care unit
9	**ACTH**	adrenocorticotropic hormone	69	**IFG**	impaired fasting glucose
10	**ADL**	activities of daily living	70	**IGT**	impaired glucose tolerance
11	**AFP**	α-fetoprotein	71	**IMT**	intima-media thickness
12	**ALP**	alkaline phosphatase	72	**LAP**	leucine aminopeptidase
13	**ALT**	alanine aminotransferase	73	**LDH**	lactate dehydrogenase
14	**Apo (a)**	apolipoprotein (a)	74	**LDL-C**	low-density lipoprotein cholesterol
15	**APTT**	activated partial thromboplastin time	75	**Lp(a)**	lipoprotein (a)
16	**AST**	aspartate aminotransferase	76	**LPL**	lipoprotein lipase
17	**BMI**	body-mass index	77	**MCH**	mean corpuscular hemoglobin
18	**CA125**	carbohydrate antigen 125	78	**MCHC**	mean corpuscular hemoglobin concentration
19	**CA19-9**	carbohydrate antigen 19-9	79	**MCV**	mean corpuscular volume
20	**cAMP**	cyclic adenosine 3', 5'-monophosphate	80	**METs**	meatbolic equivalent
21	**CAPD**	continuous ambulatory peritoneal dialysis	81	**MetS**	metabolic syndrome
22	**CBC**	complete blood cell count	82	**MMG**	mammography
23	**Ccr**	creatinine clearance	83	**MRA**	magnetic resonance angiography
24	**cDNA**	complementary deoxyribonucleic acid	84	**MRI**	magnetic resonance imaging
25	**CEA**	carcinoembryonic antigen	85	**mRNA**	messenger RNA
26	**cGMP**	cyclic guanosine 3', 5'-monophosphate	86	**MRSA**	methicillin-resistant *Staphylococcus aureus*
27	**ChE**	cholinesterase	87	**MSW**	medical social worker
28	**CKD**	chronic kidney disease	88	**NMR**	nuclear magnetic resonance
29	**COI**	conflict of interest	89	**PET**	positron emission tomography
30	**COPD**	chronic obstructive pulmonary disease	90	**PSA**	prostate-specific antigen
31	**CK**	creatinine kinase	91	**PTH**	parathyroid hormone
32	**CRP**	c-reactive protein	92	**PWV**	pulse wave velocity
33	**CT**	computed tomography	93	**QOL**	quality of life
34	**CVA**	cerebrovascular accident	94	**RBC**	red blood cell
35	**D-Bil**	direct bilirubin	95	**RF**	rheumatoid factor
36	**DBP**	diastolic blood pressure	96	**RI**	radioactive isotope
37	**DNA**	deoxyribonucleic acid	97	**RIA**	radioimmunoassay
38	**DRG**	diagnosis-related group	98	**RNA**	ribonucleic acid
39	**dsDNA**	double stranded deoxyribonucleic acid	99	**SBP**	systolic blood pressure
40	**EBM**	evidence-based medicine	100	**SD**	standard deviation
41	**ECG**	electrocardiogram	101	**SEM**	standard error of the mean
42	**eGFR**	estimated glomerular filtration rate	102	**STD**	sexually transmitted disease
43	**EIA**	enzyme immunoassay	103	**T-Bil**	total bilirubin
44	**ELISA**	enzyme-linked immunosorbent assay	104	**T_3**	triiodothyronine
45	**EPO**	erythropoietin	105	**T_4**	thyroxine
46	**ESR**	erythrocyte sedimentation rate	106	**TC**	total cholesterol
47	**FBG**	fasting blood glucose	107	**TG**	triglyceride
48	**FDA**	Food and Drug Administration	108	**TIA**	transient (cerebral) ischemic attack
49	**FEV**	forced expiratory volume	109	**TIBC**	total iron binding capacity
50	**FEV_1**	forced expiratory volume in one second	110	**tPA**	tissue plasminogen activator
51	**FEV_1%**	forced expiratory volume % in one second	111	**TPHA**	*Treponema pallidum* hemagglutination assay
52	**FPG**	fasting plasma glucose	112	**TSH**	thyroid stimulating hormone
53	**FSH**	follicle stimulating hormone	113	**TTT**	thymol turbidity test
54	**FT3**	free triiodothyronine	114	**UCG**	ultrasonic echocardiography
55	**FT4**	free thyroxine	115	**UIBC**	unsaturated iron binding capacity
56	**FVC**	forced vital capacity	116	**UN**	urea nitrogen
57	**GFR**	glomerular filtration rate	117	**VLDL**	very-low-density lipoprotein
58	**GH**	growth hormone	118	**WBC**	white blood cell
59	**Hb**	hemoglobin	119	**WHO**	World Health Organization
60	**HbA1c**	hemoglobin A1c	120	**ZTT**	zinc sulfate (turbidity) test

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The International Society of Ningen Dock, c/o Japan Society of Ningen Dock

Ningen Dock International Vol. 6 No. 1 March, 2019

Produced by
Scientist Press Co., Ltd.
5-8-10-605, Sendagaya, Shibuya-ku,
Tokyo 151-0051, Japan
TEL: +81-3-3354-2004
FAX: +81-3-3354-2017
E-mail: info@scientist-press.com

Printed by
SHINANO Co., Ltd.
4-32-8, Ikebukuro, Toshima-ku,
Tokyo 171-0014, Japan
TEL: +81-3-5911-3355
FAX: +81-3-5911-3356


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