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Association between white blood cell count and diabetes in relation to triglycerides-to-HDL cholesterol ratio in a Japanese population: The Nagasaki Islands study

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Although our previous study found that diabetes combined with a high serum triglycerides to high-density lipoprotein cholesterol (TG-HDL) ratio constitutes a risk for atherosclerosis and chronic kidney disease (CKD), the association, in terms of TG-HDL ratio, between diabetes and white blood cell (WBC) count, which is an independent risk factor for atherosclerosis, has not been clarified. To investigate this association, we conducted a cross-sectional study of 3,998 Japanese subjects aged 30-89 years undergoing a general health check. We investigated the associations between WBC count and diabetes for all subjects, who were divided into tertiles according to TG-HDL level. Independent of classical cardiovascular risk factors, WBC count of both men and women was positively associated with diabetes combined with high but not with low TG-HDL. The multivariable odds ratios (ORs) and 95% confidence intervals (95%CIs) of 1SD (standard deviation) increment in WBC count (1,538/μL for men, 1,382/μL for women) for high TG-HDL diabetes and low TG-HDL diabetes were 1.39 (95%CI: 1.04-1.85) and 0.88 (95%CI: 0.66-1.19) for men, and 1.83 (95%CI: 1.45-2.33) and 0.91 (95%CI: 0.64-1.29) for women, respectively. In conclusion, for both men and women, WBC count is associated with high TG-HDL diabetes but not with low TG-HDL diabetes. These findings suggest that measuring WBC count is clinically relevant for estimating the risk of atherosclerosis and CKD in patients with diabetes categorized according to TG-HDL ratio.

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Key words: WBC, TG-HDL, Diabetes, Cross-sectional study

Introduction

Elevated insulin concentrations are reportedly inversely associated with serum high-density lipoprotein (HDL) cholesterol concentrations[1] and positively associated with serum triglyceride (TG) concentrations.[2] Furthermore, a higher TG-HDL cholesterol ratio (TG-HDL) was found to indicate insulin resistance in general populations,[3] overweight individuals,[4] and patients with type 2 diabetes.[5] The classification of patients with diabetes according to TG-HDL levels tertiles (Shimizu's diabetes classification) in our studies[6-9] was based on the assumption that diabetes in patients with high a TG-HDL ratio is mainly caused by insulin resistance with few compensatory changes in β-cell function, while

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that of patients with a low TG-HDL ratio is mainly caused by β-cell dysfunction. Our previous studies found that categorizing patients with type 2 diabetes by TG-HDL ratio may be an effective tool for risk estimation of atherosclerosis[6] and chronic kidney disease (CKD),[8] as the presence of type 2 diabetes with a high TG-HDL ratio constitutes a risk for atherosclerosis[6] and CKD,[8] but the presence of type 2 diabetes with an intermediate and low TG-HDL ratio does not.

One study reported that a high white blood cell (WBC) count was associated with the presence of coronary heart disease, peripheral arterial disease, and stroke,[10] and another study reported that WBC count was related to early and advanced atherosclerosis independent of other risk factors.[11] Another study reported sex differences in the association between WBC count and risk for CKD;[12] no significant association was observed for men while a significant positive association was observed for women.

However, no study has been published on the associations between WBC count and type 2 diabetes categorized by TG-HDL ratios.

To investigate these associations, we conducted a cross-sectional study of 3,998 subjects (1,430 men and 2,568 women) aged 30-89 who participated in a survey on cardiovascular risk between 2005 and 2012.

Material and Methods

Subjects

Consent forms were available in Japanese to ensure comprehensive understanding of the study objectives, and written informed consent was provided by all participants. This study was approved by the Ethics Committee for Use of Humans of Nagasaki University (project registration number 0501120073).

The survey population included 4,269 participants (1,538 men and 2,731 women) aged 30 to 89 years, all residents of the western rural community of the Goto Islands. A total of 271 individuals (108 men and 163 women) with missing data were excluded, leaving 3,998 participants (1,430 men and 2,568 women) for enrolment in this study.

The mean age of the study population was 65.5 years ($\pm$10.6 SD; range 30-89) for men and 63.8 years ($\pm$11.4 SD; range 30-89) for women.

Data collection and laboratory measurements

Trained interviewers obtained information on smoking status, drinking status, medical history, use of antihypertensive agents, and use of medication for diabetes mellitus. Body weight and height were measured with an automatic body composition analyzer (BF-220; Tanita, Tokyo, Japan) at the time of drawing blood. Systolic and diastolic blood pressures were recorded at rest.

Fasting blood samples were collected in an EDTA-2K tube and a siliconized tube. Samples from the siliconized tube were used to separate the serum by centrifugation after blood coagulation. Samples from the EDTA-2K tube were used for measuring WBC count using the flow cytometry method. Serum triglycerides, serum HDL cholesterol, serum aspartate aminotransferase (AST), serum γ-glutamyltranspeptidase (γ-GTP), serum creatinine, and HbA_{1C} were measured with standard laboratory procedures.

The glomerular filtration rate (GFR) was estimated with an established method with three variations recently proposed by the working group of the Japanese Chronic Kidney Disease Initiative.[13] According to this adaptation, GFR (mL/min/1.73m^2) = $194 \times$ (serum creatinine [enzyme method])$^{-1.094} \times$ (age)$^{-0.287}$ $\times$ (0.739 for women).

HbA_{1c}, as defined by the National Glycohemoglobin Standardization Program (NGSP), was calculated with the following equation, which was recently proposed by the working group of the Japanese Diabetes Society (JDS): HbA_{1c} (NGSP) = HbA_{1c} (JDS) + 0.4%. Presence of diabetes was defined as HbA_{1c} (NGSP) $\geq$ 6.5%, and/or initiation of glucose-lowering medication or insulin therapy.[14] We further defined subtypes of diabetes by calculating tertiles of TG-HDL ratios for all the participants: low TG-HDL diabetes (median TG-HDL ratio: 1.00 for men, 0.90 for women), intermediate TG-HDL diabetes (2.08 for men, 1.77 for women), and high TG-HDL diabetes (4.37 for men, 3.59 for women) as in our previous study.[6,7]

Statistical analysis

Age-adjusted clinical characteristics were determined by least square method. TG-HDL categories for all subjects were established according to the sex-specific tertiles of TG-HDL ratios. Logistic regression models were used for calculating odds ratios (ORs) and 95% confidence intervals (95%CIs) of diabetes with TG-HDL ratios and a 1 SD (standard deviation) increment (1,538/μL for men, 1,382/μL for women) for associations with WBC count. Two different approaches were used to adjust for confounding factors. First, the data were adjusted only for age. Second, we included other possible confounding factors, namely smoking status (never smoker, former smoker, current smoker), alcohol

consumption (non-drinker, current light to moderate drinker [1-6 times/week], current heavy drinker [every day]), systolic blood pressure (mmHg), antihypertensive medication use (no, yes), history of cardiovascular disease (no, yes), body mass index (BMI [kg/m^2]), AST (IU/L), γ-GTP (IU/L), and estimated GFR (mL/min/1.73m^2).

All statistical analyses were performed with the SAS system for Windows (version 9.3; SAS Inc., Cary, NC, USA). All p-values for statistical tests were two-tailed, and values of <0.05 were regarded as statistically significant.

Results

Of the 3,998 subjects entered in this study, 302 (147 men and 155 women) were identified as having type 2 diabetes: 127 (57 men and 70 women) had high TG-HDL diabetes and 84 (48 men and 36 women) had low TG-HDL diabetes.

The age-adjusted clinical characteristics of the study population are shown in Table 1. Compared with women, men showed a significantly higher prevalence of current drinker and current smoker, significantly higher levels of γ-GTP and serum creatinine, and significantly lower levels of HDL for total subjects, participants with diabetes, and participants without diabetes. Men also showed a significantly higher prevalence of history of cardiovascular disease and higher levels of GFR for total subjects and non-diabetes participants; no such significant associations were observed for men with diabetes.

Table 2 shows the sex-specific, age-adjusted characteristics of the study populations by tertile of WBC count for all subjects. For both men and women, systolic blood pressure, diastolic blood pressure, antihypertensive medication use, BMI, current smoker, TG, and TG-HDL ratio were positively associated with WBC count, and HDL was inversely associated with WBC count. Moreover, γ-GTP was positively associated with WBC count for men, whereas serum creatinine was positively associated and GFR was inversely associated with WBC count for women.

Table 3 shows ORs and 95%CIs of diabetes and its TG-HDL subtypes in relation to WBC count. No significant association between WBC count and diabetes was observed for men, but a significant positive association was seen for women. Analysis of the association between WBC and diabetes categorized by TG-HDL level showed a significant positive association for high TG-HDL diabetes but no significant association for low TG-HDL diabetes. For intermediate TG-HDL diabetes, even multivariable OR showed no significant associations for either men or women, but women tended to show a positive association.

To exclude the influence of menopausal status, we further investigated the associations between 1 SD increments in WBC count and the risk of high, intermediate, and low-TG-HDL diabetes for elderly women (≥60 years) only and found essentially the same associations: the multivariable ORs and 95%CIs of high, intermediate, and low TG-HDL ratio diabetes were 1.82 (95%CI: 1.39-2.37), 1.30 (95%CI: 0.94-1.80), and 0.95 (95%CI: 0.64-1.42), respectively.

Table 1. Age-adjusted characteristics for study populations

	Total subjects				Diabetes				Non-diabetes			
	Total	Men	Women	p	Total	Men	Women	p	Total	Men	Women	p
No. at risk	3,998	1,430	2,568		302	147	155		3,696	1,283	2,413	
Age, years	64.4 ± 11.1	65.5 ± 10.6	63.8 ± 11.4		68.6 ± 8.7	68.5 ± 8.2	68.6 ± 9.3		64.1 ± 11.2	65.2 ± 10.8	63.5 ± 11.4	
Systolic blood pressure, mmHg	141	141	141	0.716	146	145	146	0.732	141	141	141	0.765
Diastolic blood pressure, mmHg	83	85	82	<0.001	83	83	83	0.628	83	85	82	<0.001
Antihypertensive medication use, %	30.0	27.9	31.1	0.024	40.1	33.4	46.4	0.020	29.2	27.5	30.1	0.078
Body mass index, kg/m^2	23.3	23.7	23.1	<0.001	24.3	23.9	24.6	0.108	23.2	23.7	23.0	<0.001
Current drinker, %	25.0	50.8	10.6	<0.001	25.2	43.5	7.8	<0.001	24.9	51.5	10.8	<0.001
Current smoker, %	11.2	24.6	3.6	<0.001	12.3	21.1	3.9	<0.001	11.1	25.0	3.7	<0.001
History of cardiovascular disease, %	7.5	9.4	6.4	<0.001	11.9	13.6	10.3	0.367	7.1	9.1	6.1	<0.001
Serum triglycerides (TG), mg/dL	121	126	118	0.001	137	139	136	0.742	120	125	117	0.002
Serum HDL-cholesterol (HDL), mg/dL	59	55	61	<0.001	55	52	58	0.002	59	55	62	<0.001
TG-to-HDL ratio	2.35	2.66	2.18	<0.001	2.87	3.12	2.62	0.121	2.31	2.61	2.15	<0.001
Serum aspartate aminotransferase, IU/L	23	25	22	<0.001	26	27	26	0.667	23	25	22	<0.001
Serum γ-glutamyltranspeptidase, IU/L	32	44	24	<0.001	38	46	30	<0.001	31	44	24	<0.001
Serum creatinine, mg/dL	0.77	0.90	0.70	<0.001	0.78	0.87	0.69	<0.001	0.77	0.90	0.70	<0.001
Glomerular filtration rate, mL/min/1.73m^2	69.1	70.7	68.3	<0.001	70.5	71.3	69.7	0.444	69.0	70.5	68.2	<0.001

Age: mean ± standard deviation. p: age-adjsuted p values for sex differences.

In part of our study 1,407 men and 2,541 women had data on carotid intima-media thickness (CIMT). We found that WBC count was significantly associated with risk of carotid atherosclerosis (CIMT ≥1.1 mm) for men but not for women. The age-adjusted ORs of 1 SD increments of WBC count for carotid atherosclerosis were 1.20 (95%CI: 1.05-1.38) for men and 1.04 (95%CI: 0.93-1.17) for women.

Furthermore, we evaluated the risk of CKD. No significant association was observed for men, but a significant positive association was observed for women. The age-adjusted OR for 1 SD increments of WBC count for CKD were 1.05 (95%CI: 0.93-1.19) for men and 1.25 (95%CI: 1.14-1.36) for women.

Table 2. Sex-specific relationships between age-adjusted mean values and tertiles of white blood cell (WBC) count

	WBC count tertiles			
	T1 (low)	T2	T3 (high)	p for trend
Men				
No. at risk	476	477	477	
Age, years	66.8 ± 10.6	65.5 ± 10.4	64.3 ± 10.6	
Systolic blood pressure, mmHg	140	143	143	0.009
Diastolic blood pressure, mmHg	83	85	85	0.007
Antihypertensive medication use, %	25.2	28.9	34.6	0.003
Body mass index, kg/m^2	23.1	23.8	24.2	<0.001
Current drinker, %	47.8	50.7	52.3	0.363
Current smoker, %	14.9	20.8	36.9	<0.001
History of cardiovascular disease, %	8.6	12.2	9.3	0.138
Serum triglycerides (TG), mg/dL	110	120	149	<0.001
Serum HDL-cholesterol (HDL), mg/dL	57	55	51	<0.001
TG-to-HDL ratio	2.22	2.48	3.29	<0.001
Serum aspartate aminotransferase, IU/L	25	25	25	0.394
Serum γ -glutamyltranspeptidase, IU/L	41	41	49	0.027
Serum creatinine, mg/dL	0.88	0.91	0.92	0.070
Glomerular filtration rate, mL/min/1.73m^2	71.4	69.6	69.2	0.086
Women				
No. at risk	876	836	856	
Age, years	64.0 ± 11.2	64.1 ± 11.2	63.2 ± 11.7	
Systolic blood pressure, mmHg	139	140	143	<0.001
Diastolic blood pressure, mmHg	82	82	84	<0.001
Antihypertensive medication use, %	26.0	28.0	36.7	<0.001
Body mass index, kg/m^2	22.7	23.0	23.6	<0.001
Current drinker, %	10.5	11.3	10.8	0.875
Current smoker, %	1.9	3.4	6.4	<0.001
History of cardiovascular disease, %	6.7	6.0	5.6	0.597
Serum triglycerides (TG), mg/dL	103	117	135	<0.001
Serum HDL-cholesterol (HDL), mg/dL	64	61	59	<0.001
TG-to-HDL ratio	1.84	2.15	2.57	<0.001
Serum aspartate aminotransferase, IU/L	23	22	22	0.108
Serum γ -glutamyltranspeptidase, IU/L	25	23	26	0.105
Serum creatinine, mg/dL	0.68	0.70	0.72	<0.001
Glomerular filtration rate, mL/min/1.73m^2	70.9	68.3	66.5	<0.001

Age: mean values. WBC tertiles: <5,120 /μL, 5,120-6,300 /μL, and >6,300 /μL for men and <4,930 /μL, 4,930-6,010 /μL, and >6,010/μL for women.

Table 3. Odds ratio (OR) and 95% confidence intervals (CI) for diabetes stratified by white blood cell (WBC) count

	WBC count tertiles				1 SD increment in
	T1 (low)	T2	T3 (high)	P for trend	WBC
Men					
No. at risk	476	477	477		
Diabetes					
No. of cases (%)	45 (9.5)	45 (9.4)	57 (11.9)		
Age-adjusted OR	1.00	1.04 (0.67-1.61)	1.42 (0.93-2.16)	0.097	1.07 (0.89-1.27)
Multivariable OR	1.00	1.04 (0.67-1.62)	1.45 (0.94-2.25)	0.093	1.07 (0.90-1.29)
High TG-HDL diabetes					
No. of cases (%)	12 (2.5)	14 (2.9)	31 (6.5)		
Age-adjusted OR	1.00	1.19 (0.54-2.60)	2.78 (1.40-5.49)	0.002	1.43 (1.09-1.89)
Multivariable OR	1.00	1.06 (0.48-2.36)	2.58 (1.27-5.28)	0.005	1.39 (1.04-1.85)
Intermediate TG-HDL diabetes					
No. of cases (%)	15 (3.2)	15 (3.1)	12 (2.5)		
Age-adjusted OR	1.00	1.06 (0.51-2.20)	0.89 (0.41-1.94)	0.784	0.93 (0.68-1.28)
Multivariable OR	1.00	1.04 (0.49-2.19)	0.90 (0.40-2.02)	0.802	0.93 (0.67-1.30)
Low TG-HDL diabetes					
No. of cases (%)	18 (3.8)	16 (3.4)	14 (2.9)		
Age-adjusted OR	1.00	0.93 (0.47-1.86)	0.85 (0.42-1.75)	0.664	0.84 (0.63-1.11)
Multivariable OR	1.00	1.09 (0.54-2.22)	0.97 (0.46-2.05)	0.953	0.88 (0.66-1.19)
Women					
No. at risk	876	836	856		
Diabetes					
No. of cases (%)	35 (4.0)	52 (6.2)	68 (7.9)		
Age-adjusted OR	1.00	1.60 (1.03-2.49)	2.15 (1.41-3.29)	<0.001	1.43 (1.22-1.68)
Multivariable OR	1.00	1.66 (1.06-2.61)	2.12 (1.37-3.28)	<0.001	1.41 (1.19-1.66)
High TG-HDL diabetes					
No. of cases (%)	9 (1.0)	21 (2.5)	40 (4.7)		
Age-adjusted OR	1.00	2.49 (1.13-5.47)	4.87 (2.34-10.10)	<0.001	1.90 (1.51-2.38)
Multivariable OR	1.00	2.54 (1.13-5.69)	4.61 (2.16-9.82)	<0.001	1.83 (1.45-2.33)
Intermediate TG-HDL diabetes					
No. of cases (%)	10 (1.1)	18 (2.2)	21 (2.5)		
Age-adjusted OR	1.00	1.91 (0.88-4.17)	2.26 (1.05-4.83)	0.038	1.34 (1.01-1.77)
Multivariable OR	1.00	1.95 (0.89-4.31)	2.16 (0.99-4.70)	0.059	1.30 (0.98-1.73)
Low TG-HDL diabetes					
No. of cases (%)	16 (1.8)	13 (1.6)	7 (0.8)		
Age-adjusted OR	1.00	0.85 (0.40-1.78)	0.46 (0.19-1.11)	0.089	0.80 (0.57-1.12)
Multivariable OR	1.00	1.06 (0.50-2.28)	0.60 (0.24-1.50)	0.320	0.91 (0.64-1.29)

Multivariable OR: adjusted further for age, systolic blood pressure, antihypertensive medication use, history of cardiovascular disease, body mass index, smoking, alcohol intake, serum aspartate aminotransferase, serum γ -glutamyltranspeptidase, and glomerular filtration rate. WBC count tertiles: <5,120/μL, 5,120-6,300/μL, and >6300/μL for men and <4,930/μL, 4,930-6,010/μL, and >6,010/μL for women.

Discussion

The major findings of this study were that significant positive associations independent from cardiovascular risk were observed for high TG-HDL diabetes but not for low TG-HDL diabetes in both men and women.

A previous study of ours that included 1,344 Japanese men found that diabetes combined with a high TG-HDL ratio was a risk factor for atherosclerosis (diagnosed as CIMT ≥1.1 mm) and increased arterial stiffness (diagnosed as cardio-ankle vascular index (CAVI) ≥8.0) but that intermediate and low TG-HDL diabetes were not. In that study, the multivariable-adjusted ORs and 95%CIs of atherosclerosis and increased arterial stiffness for diabetes were, respectively, 2.57 (95%CI: 1.32-5.02) and 3.56 (95%CI: 1.50-8.46) for high TG-HDL diabetes, 0.76 (95%CI: 0.29-2.00) and 0.68 (95%CI: 0.26-1.78) for intermediate TG-HDL diabetes, and 1.17 (95%CI: 0.52-2.63) and 0.80 (95%CI: 0.33-1.90) for low TG-HDL diabetes.[6] Another study with 554 subjects with primary dyslipidemia and 246 normolipidemic subjects reported that WBC count was related to early and advanced measures of atherosclerosis independent of other risk factors. The sex- and age-adjusted probability of carotid atherosclerosis and femoral plaque increased by 20% (OR, 1.20; 95%CI: 1.10-1.31) and 25% (OR, 1.25; 95%CI: 1.13-1.38), respectively, for each 1000/mm^3 WBC increment.[11] Another previous sex-specific study with 1,165 men and 2,573 women reported that WBC count was correlated with CAVI in men (β=0.61, p=0.043), but not in women (β=0.35, p=0.17).[15] This study is compatible with our present additional analysis that shows WBC count was significantly associated with carotid atherosclerosis (CIMT ≥1.1 mm) in men but not in women.

We also reported a sex-combined study (1,153 men and 1,916 women aged 60-89 years) that showed that high but not low TG-HDL diabetes constituted a significant risk for CKD (GFR <60 mL/min/1.73m^2). The adjusted ORs of classical cardiovascular risk factors for CKD were 1.52 (95%CI: 1.01-2.29) for high TG-HDL diabetes and 0.55 (95%CI: 0.31-0.97) for low TG-HDL diabetes.[8] Another study reported sex differences between associations of WBC count and risk of CKD;[12] no significant associations were seen in men while significant positive association were seen in women. This is also compatible with our present results, which showed sex differences in the association between WBC count and risk of CKD. The study reported here found further evidence that the positive associations between WBC count and diabetes were confined to high TG-HDL diabetes.

Obesity is associated with an increase in WBC count, and bariatric surgery has been shown to lower the WBC count.[16,17] Obesity causes infiltration of the visceral adipose tissue, which leads to macrophage accumulation[18] and is a major determinant of insulin resistance.[19] Insulin resistance has been found to be enhanced by the link between insulin resistance measures and WBC count, both in a general population[20] and in non-diabetic subjects.[21] Excessive adiposity increases the risk for development of a variety of pathological conditions including type 2 diabetes[22] and cardiovascular disease.[23] Furthermore, Ortega et al. reported that WBC count was associated with carotid and femoral atherosclerosis.[11] Therefore, because diabetes combined with a high, but not with a low TG-HDL ratio, is mainly caused by insulin resistance, which constitutes a risk for atherosclerosis,[6] WBC count is positively associated with high TG-HDL but not with low TG-HDL diabetes. The sex differences for the relationship between WBC count and diabetes observed in our present study might be caused by the sex differential association between intermediate TG-HDL diabetes and WBC count. For intermediate TG-HDL diabetes, even multivariable-OR showed no significant associations for either men or women, but women tended to show a positive association. Our present additional analysis showed higher prevalence of overweight (BMI >25 kg/m^2) for women than for men with intermediate TG-HDL diabetes; the age-adjusted prevalence of overweight was 44.4% for women and 33.9% for men (p=0.001). Because a high BMI is strongly associated with insulin resistance,[25] women with intermediate TG-HDL diabetes may show stronger insulin resistance. Our previous study, which reported that associations between diabetes and BMI for Japanese subjects were strongly influenced by the status of TG-HDL, might partly explain those associations.[7] Further studies to assess insulin resistance are necessary.

Some potential limitations of this study warrant consideration. First, we had no access to data for menopausal status, which can strongly influence insulin resistance in women.[24] However, when we performed our analyses only for participants aged ≥60 years, essentially the same associations were observed for women. Nevertheless, further investigations including menopausal data are needed. Second, as no data regarding exercise were available, we could not make adjustments for the effect of exercise. Finally, because this study was cross-sectional, we could not establish any causal relationships.

In conclusion, independent of classical cardiovascular risk factors, significant positive associations with WBC count were observed for both men and women with high TG-HDL diabetes but not with low TG-HDL diabetes. These

findings suggest that measuring WBC count is clinically relevant for estimating the risk of atherosclerosis and CKD in patients with diabetes categorized by TG-HDL ratio.

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