



Association between White Blood Cell Counts within Normal Range and Hemoglobin A1c in a Korean Population

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Background: We examined whether white blood cell (WBC) count levels within normal range, could be associated with hemoglobin A1c (HbA1c) levels.

Methods: Among the 11,472 people (≥ 19 years of age) who participated in the 2011 to 2012 Korea National Health and Nutrition Examination, subjects with chronic disease or illness, including 807 patients with diabetes currently taking anti-diabetic medications and/or 1,149 subjects with WBC levels $< 4,000$ or $> 10,000/\mu\text{L}$ were excluded.

Results: Overall, adjusted HbA1c levels increased across the WBC quartiles ($5.55\% \pm 0.01\%$, $5.58\% \pm 0.01\%$, $5.60\% \pm 0.01\%$, and $5.65\% \pm 0.01\%$, $P < 0.001$) after adjusting for confounding factors, such as age, gender, fasting plasma glucose, college graduation, smoking history, waist circumference, presence of hypertension, serum total cholesterol, serum triglyceride, and presence of anemia. The adjusted proportions (%) of HbA1c levels of $\geq 5.7\%$, $\geq 6.1\%$, and $\geq 6.5\%$ showed significant increases across WBC quartiles ($P < 0.001$, $P = 0.002$, and $P = 0.022$, respectively). Logistic regression analyses of WBC quartiles for the risk of HbA1c levels of $\geq 5.7\%$, $\geq 6.1\%$, and $\geq 6.5\%$, using the variables above as covariates, showed that the odds ratios of the fourth quartile of WBCs were 1.59 (95% confidence interval [CI], 1.35 to 1.89; $P < 0.001$), 1.78 (95% CI, 1.31 to 2.42; $P < 0.001$), and 2.03 (95% CI, 1.13 to 3.64; $P = 0.018$), using the first quartile of WBCs as the reference.

Conclusion: HbA1c levels were positively associated with WBC levels within normal range in a general adult population.

Keywords: Leukocyte count; Glycated hemoglobin A; Diabetes; Fasting plasma glucose

INTRODUCTION

Hemoglobin A1c (HbA1c) is used to diagnose diabetes and to identify individuals at high risk of developing diabetes or prediabetes, as well as to monitor the overall glucose control during the previous 2 to 3 months [1]. The American Diabetes Association has suggested that an HbA1c level of $\geq 6.5\%$ is the diag-

nostic cut-off point for diabetes and that a level of 5.7% is the threshold for prediabetes [2]. Measurement of HbA1c levels to screen for diabetes or prediabetes has many advantages over measuring the fasting plasma glucose (FPG) level or performing the 75-g oral glucose tolerance test (OGTT). However, HbA1c levels can be influenced by a variety of factors, including age, ethnicity, smoking, conditions that alter red cell turn-

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over, as well as glucose levels [3-8].

Several studies have shown that increased inflammation responses also influence HbA1c levels [9,10]. To date, most studies have investigated the effect of inflammation on the hyperglycemia or HbA1c levels in patients with type 2 diabetes. It is assumed that chronic inflammation impairs glucose metabolism and elevates the blood glucose concentrations, affecting higher HbA1c levels. This background has led to translation to clinical trials using anti-inflammatory drugs, investigating that inflammation might be a therapeutic target in type 2 diabetes, despite disappointing results from several studies [11,12].

Recently, a few studies have reported that HbA1c is positively associated with inflammation, even in nondiabetic subjects [13,14]. Previous study showed that an elevated leucocyte count and high-sensitivity C-reactive protein (hs-CRP) were associated with changes in HbA1c before the diagnosis of type 2 diabetes [15]. Furthermore, Liu et al. [9] demonstrated that HbA1c was positively associated with inflammation, even independent of FPG level, in nondiabetic subjects.

Although white blood cell (WBC) count is commonly measured as a reliable marker of systemic inflammation in clinical practice, few studies have investigated the clinical association of WBC level within normal range and HbA1c level, especially as a screening parameter for diabetes or prediabetes. Thus, we examined whether blood WBC levels within normal range could be associated with HbA1c levels, as a non-glycemic parameter, in a general adult population based on data from the 2011 to 2012 Korea National Health and Nutrition Examination Survey (KNHANES).

METHODS

Study population and data collection

This study is based on data from the 2011 to 2012 KNHANES, a cross-sectional and nationally representative survey conducted by the Korea Centers for Disease Control and Prevention (KCDC) [16]. The KNHANES has been conducted periodically since 1998 to assess the health and nutritional status of the civilian, non-institutionalized population in Korea. Participants were selected using proportional allocation-systemic sampling with multistage stratification. A standardized interview was conducted in the homes of the participants to collect information on demographic variables, family history, medical history, medication usage, and a variety of other health-related variables. The health interview included an established questionnaire to determine the demographic and socioeconomic characteristics of the subjects

including age, education level, occupation, income, marital status, smoking habits, alcohol consumption, exercise, previous and current diseases, and family disease history [17].

Regular exercise was defined as exercising five or more times per week. Heavy alcohol use was categorized as drinking four or more times per week during the month prior to the interview. Obesity was defined as a body mass index (BMI) ≥ 25 kg/m² according to the Asia-Pacific obesity classification [18]. Height and weight were obtained using standardized techniques and equipment. Height was measured to the nearest 0.1 cm using a portable stadiometer (Seriter, Bismarck, ND, USA). Weight was measured to the nearest 0.1 kg using a Giant-150N calibrated balance-beam scale (Hana, Seoul, Korea). BMI was calculated by dividing weight by the square of the height (kg/m²). Systolic and diastolic blood pressure (BP) was measured by standard methods using a sphygmomanometer while the patient was seated. Three measurements were recorded for all subjects at 5-minute intervals, and the average of the second and third measurements was used in the analysis.

Among the 16,576 participants who participated in the 2011 to 2012 KNHANES, 3,717 subjects under 19 years of age were excluded. Moreover, 1,387 adults did not have blood collected and were excluded. Participants with chronic disease or taking various medications that could influence even subclinical inflammation and complicate the value of WBC ($n=4,356$) were also excluded. Exclusion criteria included positivity for hepatitis B virus surface antigen ($n=405$); prior diagnosed diabetic patients with anti-diabetic medications ($n=807$), prior diagnosed hypertensive patients with anti-hypertensive medications ($n=1,839$), or anti-hyperlipidemic medications ($n=137$); a history of cancer ($n=333$); an estimated glomerular filtration rate <60 mL/min/1.73 m² ($n=315$); or a blood WBC level $<4,000/\mu\text{L}$ to exclude abnormal conditions leading to leukopenia or a blood WBC level $>10,000/\mu\text{L}$, suggesting clinically significant inflammation ($n=1,149$). Finally, data from the remaining 7,116 participants were analyzed (Fig. 1). This study included subjects who were diagnosed as hypertension (defined as systolic BP ≥ 140 mm Hg and/or diastolic BP ≥ 90 mm Hg) without anti-hypertensive medication and diabetes (defined as a FPG level ≥ 7.0 mmol/L) without anti-diabetes medication.

Laboratory methods

Blood samples were collected in the morning after fasting for at least 8 hours. Total cholesterol, FPG, triglyceride (TG), and high density lipoprotein (HDL) cholesterol levels were measured using a Hitachi Automatic Analyzer 7600 (Hitachi, To-

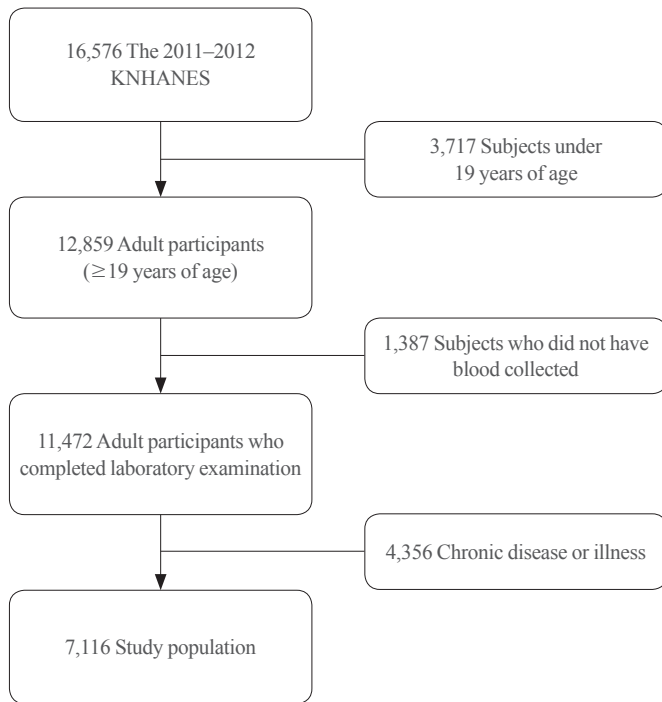


Fig. 1. Participants disposition to be included in this study. KNHANES, Korea National Health and Nutrition Examination Survey.

kyo, Japan). HbA1c was measured using high-performance liquid chromatography (HLC-723G7, Tosoh, Tokyo, Japan). WBC were quantified using an automated blood cell counter (ADIVA 120, Bayer, Tarrytown, NY, USA).

Ethics statement

This study was approved by the Institutional Review Board of Inje University Ilsan Paik Hospital, Republic of Korea (ISPAIK 2016-06-001). After approval of the study proposal, the KNHANES dataset was made available at the request of the investigator. Because the dataset did not include any personal information and participants' consent had already been given for the KNHANES, our study was exempt from the requirement for participant consent.

Statistical analyses

We classified WBC levels into the following four groups. The quartile cut-off points for the WBC level were: first quartile (Q1), 4,000 to 4,990/ μL ; second quartile (Q2), 4,991 to 5,790/ μL ; third quartile (Q3), 5,791 to 6,780/ μL ; and fourth quartile (Q4), 6,781 to 10,000/ μL .

Difference in age according to WBC quartile was evaluated with analysis of variance, and the percentage of females was

evaluated with the chi-square test. To compare age- and gender-adjusted demographics and clinical characteristics according to WBC quartile, analysis of covariance (ANCOVA) and the Bonferroni *post hoc* test were used. General linear models were used to assess HbA1c levels and the proportion (%) of HbA1c levels of $\geq 5.7\%$, $\geq 6.1\%$, and $\geq 6.5\%$ according to WBC quartile before (Model 1) and after (Models 2, 3, and 4) adjustment for confounders. Age (years), gender (men/women), and FPG level (mmol/L) were adjusted in Model 2. Model 3 adjusted for college graduation, smoking history (never, past, or current), waist circumference, presence of hypertension, serum total cholesterol, serum TG, presence of anemia (defined in accordance with the World Health Organization criteria: hemoglobin < 13 g/dL in men and < 12 g/dL in women), age, gender, and FPG level. Adjustment for confounding factors in Model 3 was performed after exclusion of FPG ≥ 126 mg/dL in Model 4. A logistic regression analysis was used to evaluate the odds ratios (ORs) of the WBC quartile for HbA1c levels of $\geq 5.7\%$, $\geq 6.1\%$, and $\geq 6.5\%$ treating age, gender, FPG, college graduation, smoking history, waist circumference, presence of hypertension, serum total cholesterol, serum TG, and presence of anemia as covariates.

The HbA1c cut-off value of 6.1% was selected because a recent Korean study showed that an HbA1c cut-off level of 6.1% was the optimal value for diagnosing diabetes, with the criteria of an FPG level of ≥ 7.0 mmol/L and/or a 2-hour FPG level of ≥ 11.1 mmol/L on the 75-g OGTT (63.8% sensitivity, 88.1% specificity) [19]. An HbA1c threshold of 5.7% had reasonable sensitivity (48.6%) and specificity (65.7%) for the identification of prediabetes [19].

All tests were two-sided, and P values < 0.05 were considered to indicate statistical significance. Statistical analyses were performed using SPSS software version 21.0 for Windows (IBM Co., Armonk, NY, USA).

RESULTS

Demographic and clinical characteristics of the study population

The demographic and clinical characteristics of the study population are shown in Table 1. The median (range) age of the population was 44 years (range, 19 to 92), and 56.1% of the participants were female. The median (range) level of WBCs was 5,790/ μL (range, 4,000 to 10,000). The mean HbA1c and FPG levels were 5.59% and 94.1 mg/dL, respectively. The percentages of subjects with HbA1c levels $\geq 5.7\%$, $\geq 6.1\%$, and

Table 1. Demographic and Clinical Characteristics of Korean Population, Aged 19 Years and Older, in 2011 to 2012 Korea National Health and Nutrition Examination Survey

Characteristic	Value
Number	7,116
Age, yr	44 (19–92)
Female sex, %	56.1
White blood cell counts, / μ L	5,790 (4,000–10,000)
College graduation, %	35.5
Heavy alcohol drinking, % ^a	6.1
Regular exercise, % ^b	7.0
Current smoking, %	21.7
Waist circumference, cm	79.9 \pm 0.1
Body mass index ^c , kg/m ²	23.4 \pm 0.1
Obesity, %	28.0
Systolic blood pressure, mm Hg ^d	116.0 \pm 0.2
Diastolic blood pressure, mm Hg ^e	75.7 \pm 0.1
Hypertension, %	13.7
Serum total cholesterol, mg/dL	191.8 \pm 0.4
Serum triglyceride, mg/dL	103.0 (17–2,327)
Serum HDL-C, mg/dL	53.3 \pm 0.1
Fasting plasma glucose, mg/dL ^f	94.1 \pm 0.2
HbA1c, %	5.6 \pm 0.1
HbA1c, mmol/mol	37.6 \pm 0.1
100 \leq FPG \leq 125 mg/dL, %	17.9
Diabetes, %	4.5
HbA1c (\geq 5.7%), %	35.2
HbA1c (\geq 6.1%), %	9.7
HbA1c (\geq 6.5%), %	3.6
Hemoglobin, g/dL ^g	14.0 (6.5–19.6)
Anemia, %	7.1
eGFR, mL/min/1.73 m ²	97.8 \pm 0.2

Values are expressed as median (range) or mean \pm SEM.

HDL-C, high density lipoprotein cholesterol; HbA1c, hemoglobin A1c; FPG, fasting plasma glucose; eGFR, estimated glomerular filtration rate.

^aHeavy alcohol drinking \geq 4 alcoholic drinks/week; ^bRegular exercise \geq 5 exercise/week; ^cBody mass index \geq 25 kg/m²; ^dSystolic blood pressure \geq 140 mm Hg without anti-hypertensive medication; ^eDiastolic blood pressure \geq 90 mm Hg without anti-hypertensive medication; ^fFasting plasma glucose \geq 126 mg/dL without anti-diabetes medication; ^gHemoglobin $<$ 13 g/dL in men and $<$ 12 g/dL in women.

\geq 6.5% were 35.2%, 9.7%, and 3.6%, respectively.

Age- and gender-adjusted demographic and clinical characteristics according to WBC quartile

Table 2 presents age- and gender-adjusted clinical characteris-

tics according to WBC quartile (Q1, 4,000 to 4,990/ μ L; Q2, 4,991 to 5,790/ μ L; Q3, 5,791 to 6,780/ μ L; and Q4, 6,781 to 10,000/ μ L). Age (Q1, 46.4 \pm 0.4 years vs. Q4, 43.9 \pm 0.4 years, P <0.001) and the percentage of women (Q1, 69.0% vs. Q4, 43.7%, P <0.001) tended to decrease with increased WBC quartile (P <0.001). There was a significant positive relationship between WBC and HbA1c levels (5.5% \pm 0.1%, 5.6% \pm 0.1%, 5.6% \pm 0.1%, and 5.7% \pm 0.1%, P <0.001), FPG level (92.5 \pm 0.4, 93.5 \pm 0.4, 94.5 \pm 0.4, and 95.8 \pm 0.4 mg/dL, P <0.001), the prevalence of impaired fasting glucose (100 \leq FPG \leq 125 mg/dL: 15.0% \pm 0.9%, 17.6% \pm 0.9%, 18.7% \pm 0.9%, and 20.3% \pm 0.9%, P <0.001), and the prevalence of diabetes (2.6% \pm 0.5%, 3.2% \pm 0.5%, 5.6% \pm 0.6%, and 6.4% \pm 0.5%, P <0.001). Systolic BP (Q1, 114.8 \pm 0.3 mm Hg vs. Q4, 117.1 \pm 0.3 mm Hg, P <0.001), diastolic BP (Q1, 75.0 \pm 0.2 mm Hg vs. Q4, 76.2 \pm 0.2 mm Hg, P <0.001), and the prevalence of hypertension (Q1, 10.9% \pm 0.8% vs. Q4, 16.9% \pm 0.8%, P <0.001) also showed significant trends across the four WBC groups. Serum total cholesterol (Q1, 186.5 \pm 0.8 mg/dL vs. Q4, 197.1 \pm 0.8 mg/dL, P <0.001), TG levels (Q1, 107.7 \pm 2.5 mg/dL vs. Q4, 155.5 \pm 2.5 mg/dL, P <0.001), and waist circumference (Q1, 78.2 \pm 0.2 cm vs. Q4, 81.6 \pm 0.2 cm, P <0.001) all increased with increased WBC quartiles. BMI (Q1, 22.8 \pm 0.1 kg/m² vs. Q4, 23.9 \pm 0.1 kg/m², P <0.001) and the prevalence of obesity (Q1, 23.0% \pm 1.1% vs. Q4, 34.8% \pm 1.1%, P <0.001) also increased with the increase in WBC quartiles. The prevalence of anemia (Q1, 9.5% \pm 0.6% vs. Q4, 5.7% \pm 0.6%, P <0.001) decreased with WBC quartile. Current smoking (Q1, 15.2% \pm 0.9% vs. Q4, 31.8% \pm 0.9%, P <0.001) was positively associated with WBC quartile. However, heavy alcohol drinking showed no association with WBC quartile.

Adjusted HbA1c (%) according to WBC quartile

We assessed the adjusted HbA1c levels according to WBC quartile and FPG level (Table 3). Overall, adjusted HbA1c levels increased across WBC quartiles (5.6% \pm 0.1%, 5.6% \pm 0.1%, 5.6% \pm 0.1%, and 5.7% \pm 0.1%, respectively; P <0.001) after adjusting for confounding factors (Models 2, 3).

Among subjects with a FPG level $<$ 100 mg/dL, HbA1c level increased along with WBC quartiles (5.4% \pm 0.1%, 5.5% \pm 0.1%, 5.5% \pm 0.1%, and 5.5% \pm 0.1%, respectively; P <0.001) before (Model 1) and after (Models 2, 3) adjustment for confounders. The adjusted HbA1c level of subjects with a FPG level of 100 to 125 mg/dL also increased along with the increase in WBC quartile (P <0.05, Models 2, 3). In Model 3, the adjusted HbA1c level (5.8% \pm 0.1%) in WBC Q1 was lower than the

Table 2. Age, Sex, and Age- and Sex-Adjusted Demographic and Clinical Characteristics According to the Quartile of WBC Level

Characteristic	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value
WBC counts, / μ L	4,000–4,990	4,991–5,790	5,791–6,780	6,781–10,000	
Number	1,795	1,765	1,777	1,779	
Age, yr	46.4 \pm 0.4	46.0 \pm 0.4	45.0 \pm 0.4	43.9 \pm 0.4	<0.001
Female sex, %	69.0	58.4	53.3	43.7	<0.001
College graduation, %	35.4 \pm 1.1	39.0 \pm 1.1	35.0 \pm 1.1	32.8 \pm 1.1	0.001
Heavy alcohol drinking, % ^a	6.0 \pm 0.6	5.6 \pm 0.6	6.3 \pm 0.6	6.7 \pm 0.6	0.562
Regular exercise, % ^b	7.8 \pm 0.6	6.9 \pm 0.6	7.3 \pm 0.6	5.8 \pm 0.6	0.145
Current smoking, %	15.2 \pm 0.9	18.1 \pm 0.9	22.0 \pm 0.9	31.8 \pm 0.9	<0.001
Waist circumference, cm	78.2 \pm 0.2	79.3 \pm 0.2	80.6 \pm 0.2	81.6 \pm 0.2	<0.001
Body mass index ^c , kg/m ²	22.8 \pm 0.1	23.2 \pm 0.1	23.6 \pm 0.1	23.9 \pm 0.1	<0.001
Obesity, %	23.0 \pm 1.1	24.2 \pm 1.1	30.0 \pm 1.1	34.8 \pm 1.1	<0.001
Systolic BP, mm Hg ^d	114.8 \pm 0.3	115.5 \pm 0.3	116.7 \pm 0.3	117.1 \pm 0.3	<0.001
Diastolic BP, mm Hg ^e	75.0 \pm 0.2	75.4 \pm 0.2	76.2 \pm 0.2	76.2 \pm 0.2	<0.001
Hypertension, %	10.9 \pm 0.8	13.1 \pm 0.8	14.1 \pm 0.8	16.9 \pm 0.8	<0.001
Total cholesterol, mg/dL	186.5 \pm 0.8	189.7 \pm 0.8	193.9 \pm 0.8	197.1 \pm 0.8	<0.001
Triglyceride, mg/dL	107.7 \pm 2.5	118.3 \pm 2.5	133.6 \pm 2.5	155.5 \pm 2.5	<0.001
HDL-C, mg/dL	54.9 \pm 0.3	53.5 \pm 0.3	52.8 \pm 0.3	52.0 \pm 0.3	<0.001
FPG, mg/dL ^f	92.5 \pm 0.4	93.5 \pm 0.4	94.5 \pm 0.4	95.8 \pm 0.4	<0.001
HbA1c, %	5.5 \pm 0.1	5.6 \pm 0.1	5.6 \pm 0.1	5.7 \pm 0.1	<0.001
HbA1c, mmol/mol	36.7 \pm 0.1	37.4 \pm 0.1	37.8 \pm 0.1	38.7 \pm 0.1	<0.001
100 \leq FPG \leq 125 mg/dL, %	15.0 \pm 0.9	17.6 \pm 0.9	18.7 \pm 0.9	20.3 \pm 0.9	<0.001
Diabetes, %	2.6 \pm 0.5	3.2 \pm 0.5	5.6 \pm 0.5	6.4 \pm 0.5	<0.001
Hemoglobin, g/dL ^g	13.9 \pm 0.1	14.0 \pm 0.1	14.2 \pm 0.1	14.3 \pm 0.1	<0.001
Anemia, %	9.5 \pm 0.6	7.6 \pm 0.6	5.6 \pm 0.6	5.7 \pm 0.6	<0.001
eGFR, mL/min/1.73 m ²	97.9 \pm 0.3	98.0 \pm 0.3	97.3 \pm 0.3	98.2 \pm 0.3	0.099

Values are expressed as mean \pm SEM.

WBC, white blood cell; BP, blood pressure; HDL-C, high density lipoprotein cholesterol; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; eGFR, estimated glomerular filtration rate.

^aHeavy alcohol drinking \geq 4 alcoholic drinks/week; ^bRegular exercise \geq 5 exercise/week; ^cBody mass index \geq 25 kg/m²; ^dSystolic BP \geq 140 mm Hg without anti-hypertensive medication; ^eDiastolic BP \geq 90 mm Hg without anti-hypertensive medication; ^fFPG \geq 126 mg/dL without anti-diabetes medication; ^gHemoglobin $<$ 13 g/dL in men and $<$ 12 g/dL in women.

HbA1c levels (5.9% \pm 0.1%) in Q3 (*post hoc* analysis, $P=0.013$) and Q4 (*post hoc* analysis, $P=0.003$) of WBC. The HbA1c levels of subjects with a FPG level of \geq 126 mg/dL showed a tendency to increase according to WBC quartile, but this tendency did not reach statistical significance.

Spearman's correlation coefficient between HbA1c and WBC were 0.116, 0.094, and 0.088 in Model 1 to 3, respectively ($P<0.001$).

The proportions (%) of HbA1c levels of \geq 5.7%, \geq 6.1%, and \geq 6.5% according to WBC quartile

The proportions (%) of subjects with HbA1c levels of \geq 5.7%,

\geq 6.1%, and \geq 6.5% are shown in Table 4 according to WBC quartile. The adjusted proportions (%) of an HbA1c level of \geq 5.7% increased across the WBC quartiles (29.0% \pm 1.0%, 32.1% \pm 1.0%, 34.8% \pm 1.0%, and 37.9% \pm 1.0%, respectively in Model 4; $P<0.001$). The adjusted proportions (%) of an HbA1c level of \geq 6.1% also increased along the WBC quartiles (5.7% \pm 0.6%, 7.1% \pm 0.6%, 8.0% \pm 0.6%, and 8.9% \pm 0.6%, respectively in Model 4; $P=0.001$). The adjusted proportion of an HbA1c level of \geq 6.5% showed a significant trend across the WBC quartiles (1.1% \pm 0.3%, 1.2% \pm 0.3%, 1.9% \pm 0.3%, and 2.0% \pm 0.3%, respectively in Model 4; $P=0.045$) after adjusting for confounders.

Table 3. Hemoglobin A1c (%) According to the Quartile of WBC Level

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value
WBC counts, / μ L	4,000–4,990	4,991–5,790	5,791–6,780	6,781–10,000	
Total					
Number	1,795	1,765	1,777	1,779	
Model 1 ^a	5.5	5.6	5.6	5.7	<0.001
Model 2 ^b	5.6 \pm 0.1	5.6 \pm 0.1	5.6 \pm 0.1	5.7 \pm 0.1	<0.001
Model 3 ^c	5.6 \pm 0.1	5.6 \pm 0.1	5.6 \pm 0.1	5.7 \pm 0.1	<0.001
Model 4 ^d	5.5 \pm 0.1	5.5 \pm 0.1	5.5 \pm 0.1	5.6 \pm 0.1	<0.001
FPG < 100 mg/dL					
Number	1,499	1,410	1,387	1,343	
Model 1 ^a	5.4	5.5	5.5	5.5	<0.001
Model 2 ^b	5.4 \pm 0.1	5.5 \pm 0.1	5.5 \pm 0.01	5.5 \pm 0.1	<0.001
Model 3 ^c	5.4 \pm 0.1	5.5 \pm 0.1	5.5 \pm 0.01	5.5 \pm 0.1	<0.001
100 \leq FPG < 126 mg/dL					
Number	262	313	332	365	
Model 1 ^a	5.8	5.8	5.9	5.9	0.061
Model 2 ^b	5.8 \pm 0.1	5.8 \pm 0.1	5.9 \pm 0.1	5.9 \pm 0.1	<0.001
Model 3 ^c	5.8 \pm 0.1	5.8 \pm 0.1	5.9 \pm 0.1	5.9 \pm 0.1	0.002
FPG \geq 126 mg/dL					
Number	34	41	58	71	
Model 1 ^a	7.4	7.7	7.6	8.0	0.403
Model 2 ^b	7.5 \pm 0.1	7.7 \pm 0.2	7.8 \pm 0.1	7.8 \pm 0.1	0.650
Model 3 ^c	7.5 \pm 0.1	7.7 \pm 0.2	7.8 \pm 0.1	7.8 \pm 0.1	0.639

Values are expressed as mean \pm SEM.

WBC, white blood cell; FPG, fasting plasma glucose.

^aModel 1: unadjusted; ^bModel 2: adjusted for age, sex, and FPG; ^cModel 3: adjusted for college graduation, smoking history (never, past, and current smoking), waist circumference, the presence of hypertension, serum total cholesterol, serum triglyceride, the presence of anemia, and variables in model 2; ^dModel 4, model 3 after exclusion of FPG \geq 126 mg/dL ($n=204$).

ORs of WBCs for the risks of HbA1c levels of \geq 5.7%, \geq 6.1%, and \geq 6.5%

We performed logistic regression analyses of WBC quartiles for the risks of HbA1c levels of \geq 5.7%, \geq 6.1%, and \geq 6.5% using age, gender, FPG, college graduation, smoking history, presence of hypertension, serum total cholesterol, serum TG, and the presence of anemia as covariates. The ORs of WBCs for HbA1c levels of \geq 5.7%, \geq 6.1%, and \geq 6.5% are shown in Table 5. Using Q1 of WBCs as the reference, Q2 to Q4 of WBCs were associated with HbA1c levels of \geq 5.7%, the current cut-off value for abnormal glucose regulation. For the HbA1c level of \geq 6.1%, using WBC Q1 as the reference, WBC Q3 and Q4 showed increased risks, by 48% ($P=0.012$) and 78% ($P<0.001$), respectively. For the HbA1c level of \geq 6.5%, using WBC Q1 as the reference, the OR of WBC Q4 was 2.03 (95%

confidence interval, 1.13 to 3.64; $P=0.018$).

DISCUSSION

Because HbA1c is formed by glycation of the N-terminal valine residue of the β -chain of hemoglobin, many conditions that affects red blood cells, such as hemolytic anemia, structural hemoglobinopathies, major blood loss, and blood transfusions, influence HbA1c levels and do so independent of glycemia [1]. However, the association between WBCs and HbA1c has received little attention.

Based on the KNHANES 2011 to 2012 data on the general adult population of Korea, we found that a higher WBC level within the normal range slightly was associated with increased the HbA1c level, and that it was so independently of the FPG

Table 4. The Proportions of HbA1c $\geq 5.7\%$, $\geq 6.1\%$, and $\geq 6.5\%$ (%) According to the Quartile of WBC Level

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value
WBC counts, / μL	4,000–4,990	4,991–5,790	5,791–6,780	6,781–10,000	
Number	1,795	1,765	1,777	1,779	
HbA1c ($\geq 5.7\%$), %					
Model 1 ^a	29.7	33.6	36.9	40.6	<0.001
Model 2 ^b	29.7 \pm 1.0	33.4 \pm 1.0	37.0 \pm 1.0	40.7 \pm 1.0	<0.001
Model 3 ^c	30.9 \pm 1.0	34.1 \pm 1.0	36.6 \pm 1.0	39.3 \pm 1.0	<0.001
Model 4 ^d	29.0 \pm 1.0	32.1 \pm 1.0	34.8 \pm 1.0	37.9 \pm 1.0	<0.001
HbA1c ($\geq 6.1\%$), %					
Model 1 ^a	6.9	9.1	10.5	12.4	<0.001
Model 2 ^b	7.6 \pm 0.6	9.3 \pm 0.6	10.3 \pm 0.6	11.6 \pm 0.6	<0.001
Model 3 ^c	7.9 \pm 0.6	9.4 \pm 0.6	10.3 \pm 0.6	11.2 \pm 0.6	0.002
Model 4 ^d	5.7 \pm 0.6	7.1 \pm 0.6	8.0 \pm 0.6	8.9 \pm 0.6	0.001
HbA1c ($\geq 6.5\%$), %					
Model 1 ^a	2.1	2.6	4.3	5.2	<0.001
Model 2 ^b	3.1 \pm 0.4	2.9 \pm 0.4	4.1 \pm 0.3	4.2 \pm 0.4	0.014
Model 3 ^c	3.1 \pm 0.4	2.9 \pm 0.4	4.1 \pm 0.4	4.1 \pm 0.4	0.022
Model 4 ^d	1.1 \pm 0.3	1.2 \pm 0.3	1.9 \pm 0.3	2.0 \pm 0.3	0.045

Values are expressed as mean \pm SEM.

HbA1c, hemoglobin A1c; WBC, white blood cell.

^aModel 1: unadjusted; ^bModel 2: adjusted for age, sex, and fasting plasma glucose (FPG); ^cModel 3: adjusted for college graduation, smoking history (never, past, and current smoking), waist circumference, the presence of hypertension, serum total cholesterol, serum triglyceride, the presence of anemia, and variables in model 2; ^dModel 4, model 3 after exclusion of FPG ≥ 126 mg/dL ($n=204$).

Table 5. ORs of White Blood Cell Quartiles for the Risks of HbA1c Levels of $\geq 5.7\%$, $\geq 6.1\%$, and $\geq 6.5\%$

	HbA1c $\geq 5.7\%$		HbA1c $\geq 6.1\%$		HbA1c $\geq 6.5\%$	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Q1	Reference		Reference		Reference	
Q2	1.20 (1.02–1.42)	0.028	1.29 (0.95–1.75)	0.109	0.85 (0.45–1.63)	0.632
Q3	1.37 (1.16–1.62)	<0.001	1.48 (1.09–2.01)	0.012	1.79 (1.01–3.18)	0.048
Q4	1.59 (1.35–1.89)	<0.001	1.78 (1.31–2.42)	<0.001	2.03 (1.13–3.64)	0.018

Covariates: age, sex, and fasting plasma glucose, college graduation, smoking history (never, past, and current smoking), waist circumference, the presence of hypertension, serum total cholesterol, serum triglyceride, the presence of anemia.

OR, odds ratio; HbA1c, hemoglobin A1c; CI, confidence interval.

level, even after adjusting for several confounding factors. This finding occurred both in the normoglycemic range with an FPG level of <100 mg/dL and in the prediabetic range with an FPG level of 100 to 125 mg/dL.

There have been a few studies showing an association between WBCs and HbA1c in subjects without diabetes. Jiang et al. [20] reported that HbA1c was independently correlated with WBC count within the normal range in subjects with and with-

out diabetes in a large Chinese population. Gustavsson and Agardh [14] showed that WBC count was independently correlated with HbA1c in non-diabetic acute coronary syndrome patients. A study using data from the US National Health and Nutrition Examination Survey also showed that polymorphonuclear leukocyte and monocyte counts independently predicted the hemoglobin glycation index in non-diabetic adults [9]. However, these studies did not assess the association of high WBC lev-

els and HbA1c levels, especially as a diagnostic parameter for diabetes or prediabetes.

This study demonstrated that a higher WBC level, even within the normal range, was associated with the HbA1c distribution, with higher proportions of HbA1c levels $\geq 5.7\%$ and $\geq 6.5\%$. Subjects in the Q4 of WBCs were about twice as likely to have diabetes as were those in the Q1 of WBC, using a diagnostic cut-off value of HbA1c $\geq 6.5\%$. This result suggests that WBC levels, even within the normal range, need to be taken into account when using HbA1c levels to screen pre-diabetes or diabetes. Thus, measuring FPG or performing the OGTT should be considered when diagnosing diabetes or prediabetes in patients in the upper normal range of WBCs and close to the diagnostic threshold of HbA1c level.

Although the mechanisms leading to increased HbA1c levels in patients with higher WBC levels even without diabetes remain unclear, insulin resistance has been suggested to have a role. Over the last decade, it has become increasingly evident that inflammation is a major component of insulin resistance [21]. Activation of proinflammatory signaling pathways that promote the differentiation and maturation of WBCs are involved in the pathogenesis of insulin resistance, via various cytokines and signaling pathways [22]. Furthermore, insulin resistance is commonly accompanied by low-grade systemic inflammation and adipose tissue inflammation [23]. Independent of initial events, the relationship becomes bidirectional in a vicious cycle [24]. Accordingly, higher WBC levels were also positively associated with parameters of insulin resistance syndrome (metabolic syndrome), such as elevated TG level, increased waist circumference, lower HDL level, hypertension, and HbA1c, in this study.

We consider other potential mechanisms, regardless of inflammation-related glucose dysregulation, because we found the association between WBCs and HbA1c to be independent of the FPG level. Recently, Liu et al. [9] reported that the correlation coefficients for the relationships between the biomarkers of inflammation and HbA1c were higher than were those for such biomarkers and FPG, suggesting that low-grade inflammation influenced variation in HbA1c levels more than did variation in FPG.

This study has several strengths. First, this is the first large population-based study to investigate the clinical association of WBC levels and HbA1c level as a screening parameter for diabetes or prediabetes. Second, patients with diabetes not currently taking anti-diabetes medications were included in this study, which differentiates it from the study populations in previous

studies. This allowed us to assess the association between WBC levels and the HbA1c distribution. Although the difference in HbA1c between subgroups of WBC quartiles was relatively small, subjects with HbA1c around 5.7% (the current cutoff value for abnormal glucose regulation criteria) and 6.5% (the diagnostic cut-off point for diabetes) can be categorized as prediabetes and diabetes or not, respectively, according to WBC level.

This study also had some limitations. First, because this was not a prospective observational study, we could not confirm any inferences regarding causality due to the cross-sectional design of the study. Second, we analyzed after adjusting for only FPG level, not including random glucose level or postprandial glucose level as confounding factors. Thus it is still uncertain whether the association between WBC level and HbA1c is mediated through elevated glucose levels caused by subclinical inflammation. Unfortunately, we could not evaluate the more accurate inflammatory marker, such as hs-CRP, reflecting subclinical inflammation, because KNHANES do not include these data.

In conclusion, HbA1c levels were positively associated with WBC levels in subjects with and without diabetes in a general adult population.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Conception or design: D.J.K., J.W.H. Acquisition, analysis, or interpretation of data: D.J.K., J.W.H. Drafting the work or revising: D.J.K, J.H.N., J.W.H. Final approval of the manuscript: D.J.K, J.H.N, J.W.H.

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