Original Article

Assessment of leukocyte count as a cardiovascular risk marker in prediabetes

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Abstract

Background and Aim: Prediabetes is reported to be associated with the cardiovascular morbidity and mortality in Indian population. This study was conducted to screen out prediabetes (impaired fasting glycemia and impaired glucose tolerance [IGT]) and to assess white blood cell (WBC) counts as a risk factor for future cardiovascular complications.

Methods: A total of 200 nondiabetic apparently healthy subjects were selected for the study. Estimation of plasma glucose (PG) levels by GOD-POD method and oral glucose tolerance test (GTT) was performed in all the subjects. Based on PG and GTT values, these subjects were divided into four groups, Group I: Normal fasting glucose/normal glucose tolerance (NFG/NGT, $n = 70$), Group II: Isolated Impaired fasting Glucose (iIFG, $n = 34$), Group III: Isolated IGT (iIGT, $n = 38$) and Group IV: Impaired fasting glucose and IGT (IFG/IGT, $n = 54$). WBC counts were estimated in all the subjects and were compared among the groups. Association of WBC with body mass index, systolic blood pressure (SBP), 2 hour post-challenge glucose levels (2 h PG) and rate pressure product was done using correlation analysis.

Results: The iIGT and IFG/IGT group had a significantly higher WBC count compared to subjects with NFG/NGT and iIFG group ($P < 0.05$). However, the WBC counts between the iIGT and IFG/IGT groups were comparable ($P > 0.05$). WBC counts were significantly associated with rate pressure product in all prediabetic subjects and with SBP and 2 h PG in subjects with IGT. Bivariate regression analysis showed association of WBC with rate pressure product (RPP) a known cardiovascular risk factor in prediabetics after adjusting other parameters.

Conclusion: This suggested that WBC count increase with raised 2 h PG in subjects with iIGT, and could be associated with metabolic syndrome and insulin resistance. Increased WBC counts and RPP in subjects with IGT, increases the cardiac risk burden in these subjects compared to subjects with IFG.

Key words: Impaired fasting glycemia, impaired glucose tolerance, white blood cell

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INTRODUCTION

Impaired fasting glycemia (IFG) and impaired glucose tolerance (IGT) are prediabetic condition which along with its fatal macrovascular complications, especially coronary heart disease (CHD) is one of the leading cause of mortality and morbidity in the Indian Population. Hence, it is important to assess the population for prediabetes by performing oral glucose tolerance test (GTT) and assessing the risk factors for future diabetes mellitus (DM) and its cardiovascular complications. Though both IFG and IGT are risk factors for future DM and CHD, IGT has been more consistently associated with metabolic syndrome increasing the risk for CHD in various studies.[1-4] However, association of IFG with future cardiovascular complications is still unclear.[1,5-7] An increased white blood cell (WBC) count even within normal range is independently associated with cardiovascular mortality in various studies.[8-10] Raised cytokines like interleukin-6 (IL-6) and IL-8 activates WBCs, which contributes to increased blood viscosity, plaque rupture, thrombus formation, and endothelial dysfunction thus increasing risk for CHD.[11,12]

Previous studies have suggested differences in lipid profile and blood pressure (BP) between IFG and IGT subjects.[2,3]

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Therefore, in this study we have compared WBC count, a marker of subclinical inflammation in subjects with IGT and IFG, to identify the high risk population. Further, correlation analysis was performed to assess the association of WBC counts with other cardiovascular risk factors such as body mass index (BMI), BP, plasma glucose (PG) levels, and rate pressure product.

MATERIALS AND METHODS

Selection of subjects

The current cross-sectional study was conducted on 200 non-diabetic apparently healthy subjects aged 25–60 years. Subjects with known cardiovascular disorders, liver dysfunction, renal dysfunction, leukemia, thyroid disorder, pregnant and nursing females, smokers and those with acute or chronic infections were excluded from the study on the basis of history and examination. A written consent was obtained from all the participants and details of procedure were explained to them in the local language. Approval from Institutional Ethics committee was obtained. Study was conducted according to the guidelines by World Medical Declaration of Helsinki.

Glucose estimation and classification of subjects

After an overnight fast, fasting blood samples were collected and 75 g oral GTT was performed in all of them at Government Medical College, Surat. Four subjects with fasting PG (FPG) >126 mg% and 2 h postchallenge glucose levels (2 h PG) >200 mg% were defined as having diabetes and excluded from the study. PG levels were estimated by GOD-POD method in all fasting and 2 h PG samples by Miura Biochemical fully automatic (Trans Asia Biomedical Limited.Model: Erba XL -640.Place: Mumbai) analyzer in Biochemistry laboratory. Based on the criteria by American Diabetic Association, the subjects were divided into four groups based on their FPG and oral GTT values as follows:

- Group I: Normal fasting glucose/normal glucose tolerance (NFG/NGT) with FPG <100 mg% and 2 h PG <140 mg%
- Group II: Isolated IFG (iIFG) with FPG >100 mg% but <126 mg% and 2 h PG <140 mg%
- Group III: Isolated IGT (iIGT) with FPG <100 mg% and 2 h PG >140 mg% but <200 mg%
- Group IV: IFG/IGT with FPG >100 mg% but <126 mg% and 2 h PG >140 mg% but <200 mg%.

Other parameters

Height and weight were measured to calculate the BMI. Heart rate (HR) and BP were measured after 10 min of supine rest. Rate pressure product,[13] which is an index of myocardial risk factor and a known cardiovascular risk parameter was calculated using the formula.

Rate pressure product (RPP) = HR × systolic BP (SBP)/100.

WBC count was determined using three part differential cell counter (SYSTMAX automated hematology analyzer) in all the subjects from their blood sample.

Statistical analysis of data

Statistical software SPSS version 16 was used for statistical analysis of data. WBC counts, BMI, FPG, 2 h PG, SBP, diastolic BP (DBP) and RPP were expressed as Mean ± standard deviation. For the purpose of statistical analysis, data were assessed for normal distribution and variables were compared in the four groups using one way ANOVA and *post-hoc* test. Pearson's correlation analysis was done to find out correlation between WBC count and other variables such as BMI, BP, RPP, and PG levels. Bivariate logistic regression analysis was done using WBC as dependent variable and RPP, a known cardiovascular risk factor as an independent variable in iIGT and IFG/IGT groups. P value < 0.05 was considered statistically significant.

RESULTS

Table 1 depicts that subjects in all the four groups were of comparable age group. There was no significant difference in BMI (except in the IFG/IGT), SBP and DBP in prediabetes subjects (IFG, IGT, and IFG/IGT) compared to NFG/NGT group. HR and RPP are significantly higher in prediabetic subjects compared to NFG/NGT group.

Table 2 depicts that WBC counts are significantly higher in iIGT and IFG/IGT groups when compared to NFG/NGT group. Moreover, both the iIGT and IFG/IGT groups had significantly increased WBC counts compared with iIFG group. Further, the WBC counts between NFG/NGT and iIFG groups, and iIGT and IFG/IGT groups were comparable.

Pearson's correlation analysis depicts the significant correlation of WBC count with RPP in prediabetic group (iIFG, iIGT and IFG/IGT groups), whereas no such correlation was found in NFG/NGT group. Furthermore, the 2 h PG values were found to be correlated with the WBC counts in both the iIGT and IFG/IGT groups and SBP in IFG/IGT group whereas no such correlation was found in other groups [Table 3].

After adjusting for BMI, SBP and 2 h PG with WBC as dependent variable and RPP as an independent variable. Findings depict that the increased WBC counts independently increases cardiovascular risk in prediabetics [Table 4].

DISCUSSION

An elevated WBC count even within the normal range is shown to be an independent risk factor for CHD in various

Table 1: Age, BMI and cardiovascular parameters among the groups

Variables	NFG/NGT	iIFG	iIGT	IFG/IGT	*P*
n	70	38	34	54	-
Age (years)	51.1±13.4	53.2±11.6	55.4±12.5	56.4±8.8	0.075
BMI (kg/m^2)	22.6±2.5	23.9±3.8	24.4±4.2	24.9±4.6**	0.006
SBP (mmHg)	119.2±9.8	120.8±8.2	122.6±8.1	122.8±10.6	0.146
DBP (mmHg)	78.6±7.9	80.6±6.6	81.1±8.1	81.8±8.6	0.138
Heart rate (bpm)	74.2±8.4	80.2±7.6***	82.6±6.3***	83.4±7.2***	0.000
RPP (mmHg/min)	89.6±6.3	95.8±6.6***	98.4±6.8***	100.4±7.1***,##	0.000

The *P*<0.05 was statistically considered significant. The * mark indicates comparison with NFG/NGT group and the # mark indicates comparison with iIFG group. **P<0.01, ***P<0.001, ##P<0.01. NFG/NGT: Normal fasting glucose/normal glucose tolerance, iIFG: Isolated impaired fasting glucose, iIGT: Isolated impaired glucose tolerance, IFG/IGT: Impaired fasting glycemia/impaired glucose tolerance, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, RPP: Rate pressure product

Table 2: FPG, 2 h PG and WBC counts among the groups

Variables	NFG/NGT (*n*=70)	iIFG (*n*=38)	iIGT (*n*=34)	IFG/IGT (*n*=54)
FPG (mg %)	93.6±4.5	116.2±5.1***	94.2±8.2###	118.4±4.3***
2 h PG (mg %)	104.4±10.2	113.4±9.1	151.2±8.6	158.4±4.2†††
WBC count (mm^3 of blood)	5960±1470	6100±1340	6880±1560*	6950±1380**,#

The P<0.05 was statistically considered significant. The * mark indicates comparison with NFG/NGT group, the # mark indicates comparison with iIFG group and † mark indicates comparision with iIGT *P<0.05, **P<0.01, ***P<0.001, ##P<0.01, ###P<0.001, †††P<0.001. NFG/NGT: Normal fasting glucose/normal glucose tolerance, iIFG:Isolated impaired fasting glucose, iIGT: Isolated impaired glucose tolerance, IFG/IGT: Impaired fasting glycemia/impaired glucose tolerance, WBC: White blood cell, FPG: Fasting plasma glucose, 2 h P: 2 hour post-challenge glucose values.

Table 3: Correlation of WBC counts with BMI, SBP, DBP, RPP, FPG, and 2 h PG among the groups

	NFG/NGT (*n*=70)		iIFG (*n*=38)		iIGT (*n*=34)		IFG/IGT (*n*=54)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
BMI	0.014	0.9	0.022	0.89	0.12	0.4	0.15	0.27
SBP	0.16	0.7	0.14	0.4	0.30	0.08	0.28	0.04*
DBP	0.06	0.62	0.15	0.36	0.12	0.49	0.14	0.31
RPP	0.22	0.06	0.33	0.04*	0.36	0.03*	0.36	0.007*
FPG	0.11	0.36	0.26	0.11	0.17	0.33	0.13	0.34
2 h PG	0.18	0.13	0.23	0.16	0.35	0.04*	0.31	0.02*

**P*<0.05 was statistically considered significant. NFG/NGT: Normal fasting glucose/normal glucose tolerance, iIFG: Isolated impaired fasting glucose, iIGT: Isolated impaired glucose tolerance, IFG/IGT: Impaired fasting glucose/impaired glucose tolerance, WBC: White blood cell, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, RPP: Rate pressure product, FPG: Fasting plasma glucose, 2 h PG: 2 hour post-challenge glucose values

Table 4: Bivariate regression analysis of WBC count with RPP in iIFG, iIGT and IFG/IGT groups

Parameters	OR (95% CI)		
	iIFG (*n*=38)	iIGT (*n*=34)	IFG/IGT (*n*=54)
RPP	1.12 (0.98-2.6)	1.54 (1.1-3.15)	1.67 (1.1-3.4)
P	0.04*	0.02*	0.01*

**P*<0.05 was statistically considered significant. CI: Confidence interval, OR: Odds ratio, WBC: White blood cell, RPP: Rate pressure product, iIFG: Isolated impaired fasting glucose, iIGT: Isolated impaired glucose tolerance, IFG/IGT: Impaired fasting glucose/impaired glucose tolerance,

studies.[8-10] Lee *et al*. associated increased WBC count with the incidence and mortality from CHD in African American population.[8] Similar association was also found in Asian and Indian population by Twig *et al*.[9] and Ganguli *et al*.[10]

Prediabetes defined using IGT and IFG have been distinctly associated with future CHD. The Fungata Diabetes study (1999),[2] DECODE study group (2001)[3] and Blake *et al*.[1] have positively associated IGT with increased risk of CHD in the western population. Similar association have been found by Pan *et al*. in Chinese population.[4] However studies associating IFG with future CHD showed conflicting results. Levitzky *et al*.[5] and Kim *et al*.[6] have positively associated IFG with CHD in women and men respectively. Whereas Blake *et al*.[1] and Hashemi *et al*.[7] have not found increased risk of CHD in subjects with IFG. Therefore, it is important to identify the high risk group in prediabetes in Indian Population so that necessary early interventions can be done to prevent future diabetes and CHD.

In the present study, an attempt has been made to evaluate and compare the leucocyte count in subjects with prediabetes (IFG and IGT) in order to identify the high risk population susceptible to future metabolic syndrome and CHD. We found that WBC counts were significantly raised in subjects with iIGT and IFG/IGT groups as compared to

those with iIFG. We have also found that WBC counts were correlated with RPP in all the three groups, with SBP in iIGT group and with SBP and 2 h PG in IFG/IGT group. By logistic regression it was observed that WBC counts have been associated with RPP which is a known cardiovascular risk factor after adjusting other parameters. Yamada *et al*. associated raised pulse pressure with increased levels of tumor necrotic factor (TNF)-alpha and neutrophil counts in elderly females.[14] WBCs activated by interleukins (6 and 8) serves as an important marker of subclinical inflammation which leads to vascular injury atherogenesis and thrombosis. WBCs also increases blood viscosity, releases products that lead to plaque rupture, thrombus formation and endothelial dysfunction thus predisposing to future risk of CHD.[11,12] Hence, in our study it has been found that iIGT and IFG/IGT groups were more prone to future risk of metabolic syndrome and CHD and require early intervention. Ohshita *et al*. also found similar rise in WBC count in IGT subjects[15] Choi *et al*. found significant rise in highly sensitive C-reactive protein (hs-CRP) in those with IGT but no significant rise in TNF-alpha and interleukins have been found in Korean population.[16] Gokulakrishnan *et al*. in CURES-64 correlated hs-CRP and raised WBC with increased risk of metabolic syndrome and CHD in subjects with NGT in the Indian population[17] Meisinger *et al*. associated hemoglobin, hematocrit and WBC count with iIGT.[18]

Limitations of the study

Other important specific markers of inflammation such as Hs-CRP, TNF alpha, fibrinogen, serum amylase, and metalloproteinase-9 have not been assessed in the present study. Therefore, future longitudinal studies should be conducted on a larger population for assessment of leukocyte count as an independent predictor of cardiovascular risk.

CONCLUSION

In the present study, we have found significantly raised WBC counts in subjects with iIGT, which was found to be associated with raised 2 h PG and RPP, an established CV risk factor. Therefore, results of this study suggest the plausible role of WBC count as a cardiovascular risk marker in identifying the high risk group among the subjects with prediabetes.

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