White blood cells and platelet profiles of diabetic patients at University of Gondar specialized referral hospital: A comparative cross-sectional study

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Abstract

Background: Altered level of many hematological parameters such as white blood cells (WBC) and platelet function has been observed in diabetes mellitus (DM) patients. Therefore, this study aimed to determine the WBC and platelet profiles and their association with anthropometric measurement and blood pressure in DM patients and healthy controls.

Method: A comparative cross-sectional study was conducted on a total of 246 participants at the University of Gondar Specialized Referral Hospital. Venous blood with K$_2$ EDTA anticoagulant was drawn and analyzed by using Sysmex KX21N hematology analyzers for WBC and platelet parameters. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 20. Results were presented as frequency and mean ± standard deviation (SD). The independent sample t test was used to compare quantitative variables between DM and control groups. The bivariate (spearman’s rank) correlation was used to analyze continuous variables. A p-value < 0.05 was considered as statistically significant.

Results: The mean platelet count was significantly higher among diabetics (252.77 ± 77.7) compared to non-diabetic controls (208.22 ± 68), p < 0.001. Similarly, the total WBC count was higher among DM patients (6.95 ± 2.23) than in the controls (6.15 ± 1.95), p = 0.04. A significant negative correlation was also found between neutrophil and duration of illness in DM patients. Besides, there is a significant positive correlation between WBC and lymphocyte number with systolic blood pressure (SBP) in DM patients.

Conclusion: Platelet and WBC count were significantly higher in DM patients than in the controls. Therefore, routine screening and profile checking of those abnormal indices is recommended to minimize DM-related complications.

Keywords
diabetes mellitus, Ethiopia, MPV, platelet indices, WBC indices
1 | INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by abnormal carbohydrate metabolism resulting in chronic hyperglycemia caused by defective insulin production or appropriate and efficient utilization of insulin by cells. DM and its complications brings a major cause of early disease and death worldwide. It has been declared an international public health issue since it has a deleterious impact on both individual and national productivity. Altered level of many hematological parameters such as WBC and the platelet function has been observed in patients with DM. Many studies have advocated the importance of the raised level of WBC count in the diagnosis of metabolic syndrome.

In diabetic states, WBCs can be stimulated by advanced glycation end products (AGE), oxidative stress, angiotensin II, and cytokines. Activated WBCs release many kinds of cytokines and transcription factors which have a fundamental role in inflammation. Furthermore, activated leukocytes can also liberate superoxide radicals and proteases that promote oxidative stress. It is expected that low-grade chronic inflammatory responses, together with other risk factors can lead to extensive vascular damage, endothelial dysfunction, and increased oxidative stress, ultimately participating in the pathogenesis of diabetic microvascular and macrovascular complications.

Blood platelets play a vital role in the blood clotting process by mediating the primary phase of hemostasis. Platelets in DM patients have been found to have increased baseline activation levels as well as enhanced stimuli-induced activation and aggregation responses. Altered platelet morphology and function have also been reported in patients with DM. There is a strong link between platelet dysfunction with platelet hyperactivity in both type-1 and type-2 DM. In DM patients, platelets have dysregulated signaling pathways that lead to an increased tendency to activate and aggregate in response to a given stimulus. Platelet abnormalities are associated with increased clotting, impaired clot breakdown, and endothelial dysfunction. This contributes to the increased risk of atherothrombotic events in people with DM compared with non-diabetic individuals.

In DM, several inflammatory markers have been studied including mean platelet volume (MPV). MPV is a simple parameter of routine hemogram assays and is a surrogate marker of platelet activation. These activated platelets, in turn, strongly related to inflammation. Since MPV increases in inflammatory conditions, it should also increase in DM because it is characterized by subclinical inflammation. An increase in MPV has also been recognized in patients with metabolic syndrome and stroke. MPV was found to be extensively higher in DM patients who are likely to be linked with the pathological processes and increased risk of vascular disease.

The MPV was significantly higher in poorly controlled diabetics compared to patients with well-controlled type-2 DM. MPV could be considered as a marker of inflammatory burden in type-2 DM and obesity. MPV is also considered to be associated with certain malignancies. Elevated MPV level might indicate malignancy in patients with thyroid nodules and lumbar disc hernias. It was found to be related to infectious diseases such as sepsis, pneumonia, and chronic prostatitis and inflammatory diseases such as ulcerative colitis. The MPV has also been studied in inflammatory diseases of the bowel. A study conducted by Aktas et al showed that MPV was increased in patients with inflammatory bowel syndrome compared to controls.

On the other hand, decreased level of MPV has been reported in some disease conditions. Even if MPV was found to be higher in most cardiovascular diseases, however, it was found to be lower in coronary artery fistula patients. Similarly, MPV values of patients with nasal polyps and Hashimoto’s thyroiditis were significantly lower than that of healthy subjects.

In the present study, we tried to compare the WBC and platelet parameters in patients with and without diabetes and also tried to find out the correlation of hematological parameters with blood pressure and anthropometric measurements.

2 | MATERIALS AND METHODS

2.1 | Study setting and design

This was a comparative cross-sectional study conducted on a total of 246 participants (164 DM and 82 controls) at the University of Gondar Specialized Referral Hospital. Gondar town is located in the central Gondar zone and is found 737 km away from Addis Ababa, the capital city of Ethiopia. The University of Gondar Specialized Referral Hospital is a teaching hospital serving approximately 5–7 million people throughout most of the Amhara regional state and nearby regions. The Diabetes illness clinic has been providing service to more than 8000 DM patients.

2.2 | Study subjects and period

The subjects were recruited for the study from January to April 2020. The inclusion criteria were diabetic patients on treatment with age >18 years and volunteer to give written informed consent. The exclusion criteria were critically ill patients, cases with a hematological disease, blood transfused patients in the last 3 months, having a history of smoking and alcoholics, pregnant women, and post-surgery patients. Volunteer non-remunerated blood donors who had no previous history of chronic diseases were included as control participants. Age and gender matching was done to eliminate selection bias. A systematic random sampling technique was used to select the study participants.

2.3 | Operational definitions

Thrombocytopenia: is considered when the platelet count becomes <150,000/mm³. On the other hand, thrombocytosis is defined when the platelet count is above 450,000/mm³.

Leukopenia: is defined as a WBC count below 4000/mm³ while, leukocytosis is considered when a WBC count is above 12,000/mm³.
BMI: Underweight is considered when BMI is <18.5 kg/m²; Normal weight: it is considered when an individual's BMI is between 18.5 and 24.9 kg/m²; Overweight is considered when an individual's BMI ranges from 25 to 29.9 kg/m²; and Obesity is considered when an individual's BMI is ≥30 kg/m².

CKD staging is done based on the kidney disease outcomes quality initiative (KDOQI) guidelines: CKD1 is considered when eGFR ≥90 and additional signs of kidney damage, CKD 2 (60–89), CKD 3 (30–59), CKD 4 (15–29) and CKD 5 (<15) ml/min 1.73 m².

2.4 | Data collection and laboratory measurements

Pretested and structured questionnaires were used to collect the sociodemographic data from the study participants. Detailed clinical history was taken from each DM patient. Anthropometric data were collected by recording the weight and height of the study participants. Weight and height were measured when the subjects were in light clothing without shoes. The BMI was calculated as weight divided by the square of height (kg/m²). Blood pressure was measured on the right arm from a sitting position following a 5 min rest. For laboratory investigation, 5 ml of venous blood with K₂ EDTA anticoagulant was drawn from each participant by vacutainer blood collection technique and analyzed by using Sysmex KX21N analyzers for hematological parameters like WBC count, neutrophil, lymphocytes, MPV, and platelet count.

2.5 | Ethical declaration

Ethical clearance was obtained from the ethical Review Committee of the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar. Written-informed consent was obtained from the study participants prior to the start of the study. The study was also done following the declaration of Helsinki.

2.6 | Statistical analysis

Data were analyzed using SPSS version 20 (IBM Corporation). The results were expressed as frequency and mean ± SD. The independent sample t test was the test of significance for quantitative variables between two groups. The data were tested for normality with the help of Shapiro–Wilk’s and Kolmogorov–Smirnov’s tests. The bivariate correlation was used to analyze continuous variables. A p-value < 0.05 was considered as statistically significant.

3 | RESULT

3.1 | Sociodemographic characteristics

A total of 246 study participants (164 DM patients and 82 healthy controls) were enrolled in this study. Of the total participants, 144 (96 DM and 48 controls) and 102 (68 DM and 34 controls) were male and female, respectively. The mean age of the DM patients and controls were 48.23 ± 15.3 and 46.5 ± 11.4, respectively. One-hundred seventy (69.1%), 44 (17.4%), 16 (6.5%), 16 (6.5%) were married, single, widowed, and divorced, respectively.

3.2 | Behavioral and clinical characteristics of the study participants

The study revealed that 7 (4.3%) and 69 (42.1%) of the participants had a history of cigarette smoking and alcohol use, respectively. Seventy-four (45.1%) DM patients had <5 years duration of illness. Eighty-two (50%) of the DM patients were overweight; moreover, hypertension (27.4%) and kidney disease (14.6%) were the common comorbidities in those patients. In this study, 11 (6.9%) had an estimated glomerular filtration rate (eGFR) value of <60 ml/min/1.73 m² (Table 1).

3.3 | Comparison of the WBC and platelet parameters of the study participants

The mean value of platelet count was 252.77 ± 77.7 and 208.22 ± 68 among DM and controls, respectively. WBC, lymphocyte number, and the number of mixed cells were found significantly higher in DM patients as compared to the controls. However, there was no statistically significant difference between the diabetic and non-diabetic groups respecting platelet distribution width (PDW), MPV, platelet large cell ratio (P-LCR), lymphocyte percent, neutrophil percent, and percent of mixed cells (Table 2).

3.4 | Hematological abnormalities (WBC and platelets) among DM patients

Of the total 164 DM patients, 12 (7.3%) and 5 (3.0%) had thrombocytopenia and thrombocytosis, respectively. On the other hand 12 (7.3%) and 18 (11%) DM patients had leucopenia and leucocytosis, respectively.

3.5 | Correlations of WBC and platelets with BMI, duration of illness, and blood pressure

Among the platelet parameters, only platelet (p-value = 0.021) was found positively correlated with BMI in DM patients. Among the WBC parameters, neutrophil has a significant negative correlation with duration of illness in DM patients. On the other hand, mixed cells have a significant positive correlation with the duration of illness (Table 3).

Among the WBC parameters, only WBC (p-value = 0.049) and lymphocyte number (p-value = 0.004) were found positively correlated with SBP in DM patients. No significant correlation was observed among platelet parameters with SBP and diastolic blood pressure (DBP) (Table 4).
The present study compares the hematological parameters between patients with DM and healthy controls. The study showed statistically greater platelet count among DM patients than in the controls. This finding is following previous studies done in Nigeria, Ethiopia, Turkey, and Brazil.

Platelets of patients with DM are characterized by deregulation of several signaling pathways and have been proven to be hyper-reactive with intensified adhesion, activation, and aggregation. Such a hyper-reactive platelet phenotype may contribute to the higher proportion of DM patients with inadequate response to antiplatelet agents compared with non-DM subjects. Due to microhemorrhages in the atheromatous plaques, the bone marrow is signaled to release reserve and immature giant platelets. In DM patients, platelets have increased expression of adhesion molecules. A study by Eibl et al. showed that platelets from DM patients have a
greater expression of platelet activation markers compared with an age-matched non-diabetic control group.

Several mechanisms are suggested to increased platelet reactivity observed in DM patients. These mechanisms include hyperglycemia, insulin deficiency and resistance, associated metabolic conditions (obesity, dyslipidemia, and increased systemic inflammation), and other cellular abnormalities. Patients with type 2 DM are prone to have dyslipidemia, typically manifested by elevated levels of triglycerides. A high level of triglyceride is a typical manifestation that is known to induce higher platelet activation. An increase in reactive oxygen species in addition to chronic hyperglycemia enhances the production of AGEs. These products may contribute to the development of atherosclerotic complications by activation of the receptor for AGEs and by enhancing platelet aggregation through the serotonin receptor. A study done by Taniguchi et al. in Japan has indicated that increased platelet count may independently predict insulin resistance among type-2 DM patients.

Statistically significant association was not found in the MPV value among DM patients and controls in the present study. Similarly, no significant association was found between MPV and type-II DM, myocardial infarction patients, and acute coronary syndrome.

In the present study, WBC indices (WBC count, lymphocyte, and mixed number) increased significantly in the DM patients compared with the control group. This study supports the finding of previous studies conducted in Libya, Ethiopia, India, Nigeria, Bangladesh, and Turkey. Alteration in WBC indices has also been reported in DM patients. Higher WBC count has been described as an indicator of chronic inflammation which is associated with microvascular complications in type 2 DM. Also, Elevated WBC count even within the normal range has been reportedly associated with micro and macrovascular complications. Evidence from epidemiological studies suggests an association between the WBC count, a non-specific marker of inflammation, and diabetes risk.

A significant negative correlation was found between neutrophil and duration of illness. Numerous studies have shown that in DM, many alterations in neutrophil function might contribute to the high prevalence of infections in those patients. Further, the extreme

### Table 3: Spearman’s correlations (rho) of WBC and platelet parameters with BMI and duration of illness among DM patients and healthy controls at University of Gondar Specialized Referral Hospital, Northwest Ethiopia, 2020

| Parameters | DM BMI rho (p-value) | Control BMI rho (p-value) |
|------------|----------------------|---------------------------|
| Platelet   | 0.181 (0.021)        | -0.053 (0.501)            |
| PDW (fl)   | 0.049 (0.542)        | 0.087 (0.283)             |
| MPV (fl)   | 0.052 (0.512)        | 0.115 (0.146)             |
| P-LCR %    | 0.053 (0.503)        | 0.107 (0.186)             |
| WBC (10⁹/µl) | -0.073 (0.350)    | -0.101 (0.200)            |
| Neutrophil % | -0.053 (0.508)   | -0.163 (0.041)            |
| Lymphocyte % | 0.066 (0.404)     | 0.072 (0.362)             |
| Mixed %    | -0.101 (0.207)      | 0.237 (0.003)             |
| Neutrophil # | 0.011 (0.889)      | -0.134 (0.093)            |
| Lymphocyte # | 0.135 (0.088)     | -0.004 (0.956)            |
| Mixed #    | -0.034 (0.676)      | 0.136 (0.096)             |

*Correlation is significant at the 0.05 level (2-tailed); rho = spearman’s correlation coefficient.

### Table 4: Spearman’s correlations (rho) of WBC and platelet parameters with SBP and DBP among DM patients and healthy controls at University of Gondar Specialized Referral Hospital, Northwest Ethiopia, 2020

| Parameters | DM SBP rho (p-value) | DM DBP rho (p-value) | Control SBP rho (p-value) | Control DBP rho (p-value) |
|------------|----------------------|----------------------|---------------------------|---------------------------|
| Platelet   | 0.005 (0.953)        | -0.020 (0.798)       | -0.053 (0.635)            | -0.149 (0.150)            |
| PDW        | 0.028 (0.729)        | -0.035 (0.669)       | 0.026 (0.845)             | 0.092 (0.498)             |
| MPV        | 0.086 (0.278)        | 0.028 (0.728)        | -0.114 (0.397)            | -0.019 (0.888)            |
| P-LCR %    | 0.058 (0.475)        | 0.019 (0.811)        | -0.154 (0.258)            | 0.013 (0.926)             |
| WBC        | 0.154 (0.049)        | 0.077 (0.355)        | -0.050 (0.659)            | 0.045 (0.687)             |
| Neutrophil | -0.009 (0.912)       | 0.014 (0.865)        | 0.056 (0.705)             | 0.093 (0.531)             |
| Lymphocyte | 0.084 (0.285)        | -0.013 (0.867)       | -0.034 (0.767)            | 0.027 (0.814)             |
| Mixed      | -0.044 (0.578)       | 0.021 (0.794)        | 0.072 (0.625)             | 0.013 (0.928)             |
| Neutrophil # | 0.110 (0.166)      | 0.089 (0.262)        | -0.093 (0.533)            | 0.03 (0.986)              |
| Lymphocyte # | 0.224 (0.004)      | 0.082 (0.301)        | 0.019 (0.885)             | 0.135 (0.307)             |
| Mixed #    | 0.034 (0.675)        | 0.036 (0.656)        | -0.052 (0.733)            | -0.048 (0.750)            |

*Correlation is significant at the 0.05 level (2-tailed); rho = spearman’s correlation coefficient.
production of cytokines may lead to inappropriate activation and tissue damage and even increase vulnerability to invasive microorganisms. Therefore, the increased neutrophil responsiveness in DM may be considered as a part of diabetic physiopathology.\textsuperscript{51}

Our data showed a significant positive correlation between WBC count and lymphocyte number with SBP in DM patients. The correlation between the level of Blood pressure and hematological parameters might be a result of the development of DM-related hypertension and dyslipidemia in those patients.\textsuperscript{52} This might also be explained by the fact that AGEs formed in diabetes quench nitric oxide (NO) in vitro and may reduce the vasodilatation effect of NO. It also induced the production of the vasoconstrictor endothelin-1 by endothelial cells through nuclear factor-kB activation. Therefore, since endothelin-1 is a potent vasoconstrictor, it increases systemic blood pressure.\textsuperscript{53} In our study, platelet count achieved a significant correlation with BMI. A study done by Ganz et al.\textsuperscript{54} suggested that BMI is strongly and independently associated with the risk of T2DM. Since the BMI is highly associated with hypertension, hypertriglyceridemia, and hypercholesterolemia, fat distribution has been described as an important marker of inflammation and is directly linked to changes in cytokine concentrations and platelet count.\textsuperscript{55}

The present study had a few limitations of being less in sample size; a large clinical trial is needed to confirm the present study findings. Our study is cross-sectional, and thus it is not possible to discern cause and effect using this design.

5 | CONCLUSION

Platelet and WBC count were significantly higher in DM patients than in the controls. Therefore, routine screening and treatment of those abnormal indices is recommended to minimize DM-related complications.

CONFLICTS OF INTEREST

None declared.

AUTHOR CONTRIBUTION

TA and SG conceived the study, data collection, data analysis, and original draft writ-up. FA and ZG participate in data analysis and reviewed the initial and final drafts of the manuscript. All authors read and approved the final manuscript. All the authors critically revised the paper and agreed to be accountable for all aspects of the work.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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