

Analysis of White Blood Cells Morphology and Neutrophils to Lymphocytes Ratio of Type 2 Diabetes Mellitus Patients

Ngwu Amauche Martina

Department of Medical Laboratory Science,
Faculty of Allied Health Sciences,
Enugu State University of Science and Technology,
Enugu, Nigeria.

amauche.ngwu@esut.edu.ng

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Abstract

Hyperglycemia increases stress to the body and makes the body system more or less complicated. This includes white blood cells (WBC) of the immune system. This study aimed to determine the WBC morphology and neutrophil-to-lymphocyte (N/L) ratio of type 2 diabetes mellitus (T2DM) patients. A case-control study was conducted at Enugu State University Teaching Hospital (ESUTH) from July to November 2024, involving 70 T2DM patients (aged 40–60 years) and 30 age-matched healthy controls. Total white blood cell (TWBC) count and differential count such as neutrophils, lymphocytes, monocytes, eosinophils and basophils were measured using an automated analyzer. Peripheral blood smears were also prepared and examined for morphological abnormalities. Neutrophil-to-lymphocyte ratio and monocytes –to-lymphocytes (M/L) ratio were calculated from differential count. The mean lymphocytes was significantly higher among T2DM ($55.58 \pm 8.15\%$) compared to non-diabetic subjects ($40.62 \pm 4.79\%$), $p < 0.001$. The mean neutrophil/lymphocyte ratio and neutrophil count was significantly lower in the T2DM group compared to control group ($p < 0.001$). Peripheral blood smear examination revealed predominantly lymphocytes in 52% of patients and left shift of neutrophil in one female subject. A significant positive correlation was also found between neutrophil and N/L ratio in T2DM. T2DM decreased neutrophil/lymphocyte ratio, increased lymphocytes, morphological changes including left shift of neutrophils. Based on findings, routine examination of white blood cell is recommended to minimize diabetes related complications.

Keywords: *neutrophil/lymphocyte ratio, monocytes, type 2 diabetes mellitus*

Introduction

Type 2 diabetes is major causes of morbidity and mortality, and their prevalence is increasingly rising in the middle age population. In type 2 diabetes, noticeable changes have been observed in immune system components such as macrophages and T-cells. The leading changes occur in white blood cells, in association with the adipose tissue, pancreas, liver, vascular system, and circulation. These immunological changes alter the levels of individual cytokine (Calle and Fernandez, 2012; Donath and Shoelson, 2011; Chang and Halter, 2003). White blood cells are part of the immune system and take part in innate and humoral immune responses. They move around in the blood and increases inflammatory and cellular responses to injury or pathogens (Grange and Senchenkova, 2010; Medzhitov, 2008). Study had shown that inflammatory cells invade the adipose tissue in obese individuals, connected with increased formation and secretion

of inflammatory cytokines that may add to entire body inflammation (Blüher, *et al* 2009; Wellen and Hotamisligil, 2003). However, chronic inflammation has been linked with an increased prevalence of diabetes even when there is no obesity (Han, *et al* 2006; Solomon, *et al* 2010), such has been reported in patients with psoriasis and rheumatoid arthritis, and management with anti-inflammatory drugs in these conditions significantly reduced the rates of diabetes Goldbach-Mansky (2009). Some epidemiological studies have shown association between various markers of inflammation and development of diabetes, including white blood cell counts, neutrophils, lymphocytes, monocytes, and C-reactive protein (Dehghan, *et al* 2007; Duncan, *et al* 2003; Festa, *et al* 2002; Barzilay, *et al* 2001). A non-specific marker of inflammation, such as white blood cell has also been demonstrated to be linked with development of diabetes in some groups (Nakanishi, *et al* 2003; Vozarova, *et al* 2002; Schmidt, *et al* 1999). The white blood cells (WBCs) move through the capillaries with a diameter smaller than their own size. The WBCs move through the endothelium, through the process of leukocyte adhesion and enter through the process of leukocyte transmigration McEver (2005). The processes depend on the leukocyte deformation (Lee, *et al* 2011; Geert, 2008). Blood cell deformation could be determined by following three biological factors: (1) the morphological feature of blood cells such as diameter, volume, concave depth and surface area (2) the adhesive coefficient of cells, and (3) the elastic effects of blood cell membranes (Kostova, *et al* 2015; Tran-Son-Tay and Nash, 2007; Stoltz, *et al* 1999; Wautier, *et al* 1999). White blood cells are rigid than the red blood cells, therefore not only erythrocytes could block microcirculation and blood flow but leucocytes are also involved Bagge, *et al* (1981). In addition, the damaged WBCs in diabetes might interrupt capillary flow in the kidney and retina Barnes, *et al* (1981). Therefore, we supposed that the evaluation of the morphological characteristics of the leukocyte structure from patients with T2DM, could show changes in the cell type, such as granulocytes or lymphocytes, and to identify any associated hematologic conditions. New inflammatory markers obtained from standard blood count test are in demand nowadays. One of these derived new inflammatory markers is neutrophil to lymphocyte ratio, which is obtained by simply division of neutrophil count by lymphocyte count in hemogram. It shows high inflammatory significance of certain diseases Chung, *et al* (2007). Numerous studies had reported that NLR was significantly high in subjects with chronic obstructive pulmonary disease compared to the healthy individuals. Recent studies have found significant association between neutrophil to lymphocyte ratio and several conditions (Ohshita, *et al* 2004; Vozarova, *et al* 2002; Jones, *et al* 198). The aim of the present study was to evaluate WBC morphology and neutrophil-to-lymphocyte (N/L) ratio of type 2 diabetes mellitus patients.

2.0 Materials and Methods

2.1 Study subjects

This study consisted of 100 subjects (ages ranging from 40-60 years) who were divided into two groups. The first group consisted of diabetic patients (n=70; males-31, females-39) and the second group consisted of non-diabetic individuals (n=30; males-10, females-20). The study was carried out during the period of July to November 2024 in the Department of Hematology and the Diabetes Outpatient Clinic of Enugu State University Teaching Hospital, Enugu, Nigeria.

Ethics approval and consent

The study was conducted after ethics approval from the Ethical Committee of Enugu State University Teaching Hospital, and all participants signed an informed consent form before the study commenced.

Laboratory evaluations

The subjects were requested to fast overnight. Blood samples were collected by venipuncture from diabetic and non-diabetic subjects. For making of blood smear and estimation of complete blood count, 3 mL of blood was taken in a separate test tube containing ethylene di-amino tetra acetic acid (EDTA). Blood counts (including TWBC and differential count) were performed in an automatic counting machine (Mindray BC 5150). Peripheral blood smears were prepared, air-dried, and stained with Leishman stain according to standard protocols Cheesbrough (2006). Morphological abnormalities such as left shift of neutrophils were graded according to published criteria (Shah, *et al* 2020; Rozenberg, 2011). However, for glucose testing, 2mL of blood was taken into a separate test tube containing fluoride fluid and within one hour of sample collection, plasma was separated.

Statistical Analysis

Statistical Package for Social Sciences version 25.0 was used to perform the statistical analyses. The data were expressed as mean $\pm$ SD or expressed as %. The mean values were tested for significance using a test for paired samples. Pearson's correlation coefficients were used to determine the relationship between N/L ratio, M/L ratio and differential counts. The values were regarded as statistically significant when $P < 0.05$.

Result

The mean neutrophils count and N/L ratio were significantly lower in T2DM patients compared with controls ($39.87 \pm 8.79\%$ vs. $54.61 \pm 6.53\%$, $p < 0.001$ and 0.79 ± 0.58 vs. 1.38 ± 0.34 , $p < 0.001$). The mean lymphocytes count was significantly high in T2DM patients compared with controls ($55.58 \pm 8.15\%$ vs. $40.62 \pm 4.79\%$, $p < 0.001$). However, M/L ratio showed no significant differences between groups (Table 3.1).

Table 3 1: white blood cells (WBC) Parameters of T2DM Patients vs. Controls

Parameters	test (N=70)	control (N=30)	p-value
TWBC ($10^9/L$)	6.26 ± 2.72	5.92 ± 1.19	0.65
Neutrophils (%)	39.87 ± 8.79	54.61 ± 6.53	$<0.001^{**}$
Lymphocytes (%)	55.58 ± 8.15	40.62 ± 4.79	$<0.001^{**}$
Monocytes(%)	2.07 ± 0.94	1.67 ± 0.61	0.03*
Eosinophils (%)	2.38 ± 2.29	2.44 ± 1.70	0.98
Basophils (%)	0.14 ± 0.06	0.10 ± 0.01	0.99
N/L	0.79 ± 0.58	1.38 ± 0.34	$<0.001^{**}$
M/L	0.04 ± 0.02	0.04 ± 0.01	0.65
Age (years)	49.99 ± 7.99	26.87 ± 3.81	$<0.001^{**}$

The mean FBS was significantly high in Male patients compared with female patients (7.30 ± 1.05 mmol/L vs. 6.45 ± 0.92 , $p = 0.002$). Other hematological indices, including TWBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils, N/L and M/L showed no significant differences between groups (Table 3.2).

Table3.2: Gender-Based Comparison

Parameters	male (N=31)	female (N=39)	p-value
TWBC ($10^9/L$)	6.17 ± 2.49	6.34 ± 2.90	0.96
Neutrophils (%)	41.20 ± 5.46	38.86 ± 10.59	0.46
Lymphocytes (%)	53.83 ± 4.34	56.89 ± 9.97	0.20
Monocytes (%)	2.17 ± 0.99	1.99 ± 0.89	0.72
Eosinophils (%)	2.36 ± 2.00	2.38 ± 2.18	1.00
Basophils (%)	0.15 ± 0.07	0.13 ± 0.05	0.63
N/L	0.77 ± 0.15	0.79 ± 0.76	0.99
M/L	0.04 ± 0.02	0.04 ± 0.02	0.81
FBS (mmol/L)	7.30 ± 1.05	6.45 ± 0.92	0.002*

Among the WBC parameters, neutrophils (p -value = <0.001, 0.004) were found positively correlated with N/L ratio and M/L ratio in T2DM patients while lymphocytes (p -value = <0.001, 0.001) were found negatively correlated with N/L ratio and M/L ratio in T2DM patients. No significant correlation was observed among other WBC parameters with N/L and M/L (Table 3.3).

Table 3.3: Pearson's correlations (r) of TWBC and differential counts with N/L ratio and M/L ratio among T2DM patients and controls.

Parameters	T2DM		control	
	N/L ratio r (p -value)	M/L ratio r (p -value)	N/L ratio r (p -value)	M/L ratio r (p -value)
TWBC	0.096 (0.428)	0.078 (0.523)	0.118 (0.535)	0.287 (0.124)
Neutrophils	0.804 (0.000)**	0.342 (0.004)**	0.933 (0.000)**	0.176 (0.352)
Lymphocytes	-0.804 (0.000)**	-0.463 (0.000)**	-0.952 (0.000)**	-0.263 (0.160)
Monocytes	-0.053 (0.662)	0.773 (0.000)**	-0.086 (0.652)	0.937 (0.000)**
Eosinophils	-0.165 (0.171)	0.047 (0.701)	-0.120 (0.528)	0.010 (0.958)
Basophils	-0.090 (0.460)	-0.158 (0.631)	-0.155 (0.414)	-0.019 (0.919)
Age (years)	-0.088 (0.471)	-0.112 (0.354)	0.114 (0.550)	-0.141 (0.456)

In the blood film of male T2DM subjects, 26 (83.9 %) showed predominantly lymphocytes and 3 (9.7%) had mild lymphocytosis. In the blood film of female T2DM subjects, 26 (66.7%) showed predominantly lymphocytes, 7 (17.9%) showed mild lymphocytosis, while 4 (10.3%) marked lymphocytosis were seen in blood film. However, one blood film (2.6%) of female subjects showed neutrophilia with mild left shift of neutrophils (Figure 3.1).

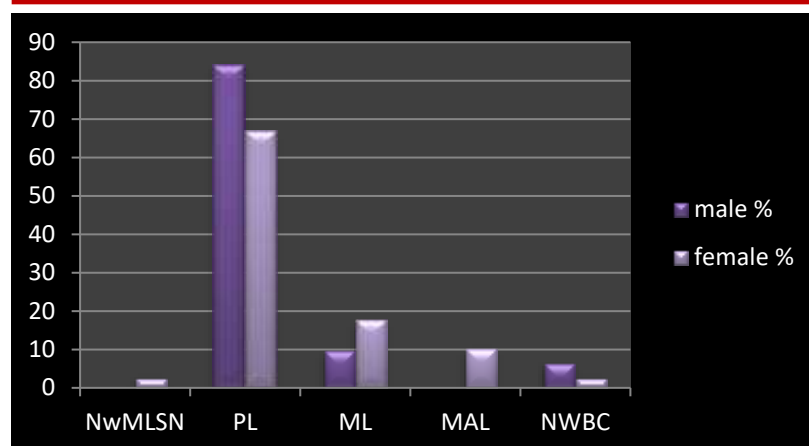


Fig 3.1: WBC morphology of male and female T2DM subjects

Abbreviation: NwMLSN (neutrophilia with mild left shift of neutrophils), PL (predominantly lymphocytes), ML (mild lymphocytosis), MAL (marked lymphocytosis), NWBC (normal WBC). In the blood film of T2DM subjects, 52 (74.3 %) showed predominantly lymphocytes, 10 (14.3%) showed mild lymphocytosis, 4 (5.7%) showed marked lymphocytosis, while 3 (4.3%) showed normal white blood cell morphology. However, one blood film (1.4 %) of T2DM subjects showed neutrophilia with mild left shift of neutrophils. In the blood film of non-diabetic subjects, 9 (30%) showed predominantly lymphocytes, while 21 (70%) showed normal white blood cell morphology (Figure 3.2)

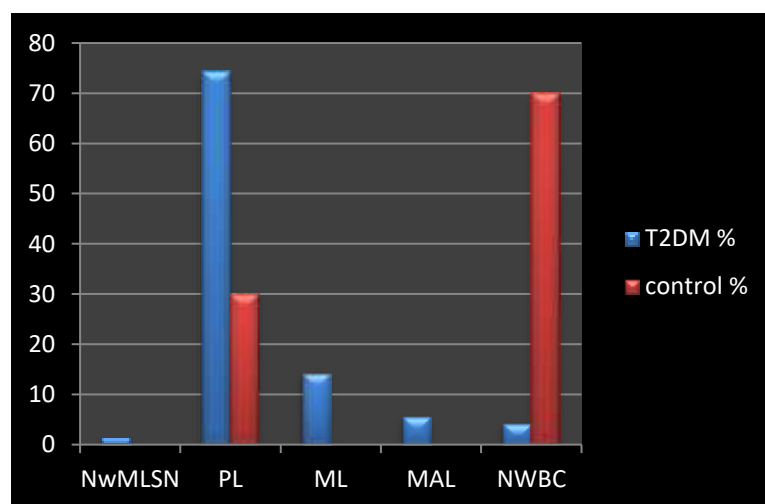


Fig 3.2: WBC morphology of T2DM and non-diabetic control subjects

Discussion

Present study showed that NLR levels were decrease in patients with T2DM. This decrease is probably associated with favorable prognostic factors, reflecting a maintained immune balance. Study on the association between the neutrophil-to-lymphocyte ratio and type 2 diabetes mellitus shown that NLR levels of T2DM patients were significantly higher than non-T2DM patients Chen, *et al* (2024). However, results of the present case control study slightly differ from result of previous studies on type 2 diabetes mellitus (Medugu, *et al* 2025; Varma, *et al* 2021). In 2020,

Wu and Gao, showed decreased lymphocyte count in patients with T2DM. Another study from Chinese reported total WBC count, neutrophil count and lymphocyte count were significantly increased in subjects newly diagnosed with diabetes mellitus compared to non-DM subjects (Zhang, *et al* 2017). Increased levels of lymphocytes are one of the predictors for incidence of type 2 diabetes. In agreement with literature, the present study observed higher lymphocytes count in type 2 diabetes mellitus than non-T2DM patients. A moderate negative correlation between lymphocytes counts and NLR has been reported (Sukrisman, *et al* 2021). The role of NLR as a flag of immune system homeostasis is well established. NLR, an emerging marker of diseases has a well-known prognostic value and independently correlates with mortality in the general population and in several specific subgroup of disease including T2DM, sepsis, COVID-19, pneumonia, cancer (Buonacera, *et al* 2022; Liu, *et al* 2021; Cataudella, *et al* 2017; De Jager, *et al* 2010). In this finding, 74.3% of the T2DM patients had predominately lymphocytes in their blood film with 1.4% neutrophilia with mild left shift of neutrophils. This is similar to a study done in University of Gondar Comprehensive Specialized Hospital which report that Neutrophilia and lymphocytosis were the common white blood cell abnormalities detected in 53.43% of the patients Getawa and Adane, (2022). This could be as a result of abnormal neutrophil yield and maturation, peripheral consumption or damage, and tissue detainment. In the present study, mild lymphocytosis was found in 14.3% of patients, while marked lymphocytosis was found in 5.7% and normal white blood cell (WBC) morphology was found in 4.3% of the patients. However, lymphocytosis is a usual hematologic abnormality in adults with mainly reactive and sometimes malignant causes (Devi, *et al* 2022; Strati and Shanafelt, 2015; Parikh, *et al* 2013). The result of the present study was slightly in line with other results which have shown that differential counts of diabetic patients had polymorph in range of 61-70 percent and polymorphonuclear leukocyte was above 70% MNDJ and DK, (2017). When diagnosed properly, NLR, WBC count/morphology can be a vital tool for laboratory hematology professionals to recommend proper laboratory follow-up and to determine the best tests for definitive diagnosis

Conclusion

This study demonstrated that patients with type 2 diabetes mellitus show decreased neutrophil/lymphocyte ratio, increased lymphocytes, morphological changes including left shift of neutrophils. Based on findings, routine examination of white blood cell is recommended to minimize diabetes related complications.

Conflict of interests

Authors declared that no competing interests exist

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