Research Article

Evidence for Parainflammation According To Differential Leukocyte Ratios in a Cohort of Type 2 Diabetic Mellitus Patients

Alphonsus Ogbonna Ogbuabor¹, Mabel Chika Ogbuabor², Ibe Chinwe Onyeka³

¹Department of Medical Laboratory Sciences, College of Medicine, Enugu State University of Science and Technology, Enugu State, Nigeria.
²Department of Medical Laboratory Sciences, Ebonyi State University, Ebonyi, Nigeria
³Department of Medical Laboratory Sciences, Nnamdi Azikiwe University, Anambra State Nigeria, Department of Medical Laboratory Sciences, Evangel University Akaeze, Ebonyi State Nigeria.

Abstract:

Diabetes mellitus has over the years become a public health and a complex disease. It is characterized by chronic hyperglycemia. The present study was designed to determine the differential leukocyte ratios in type 2 diabetic patients compared to non-diabetic controls. A total of 240 subjects comprising 120 type 2 diabetic patients (60 males and 60 females) aged 20-25 years and 120 apparently healthy age and gender-matched controls were recruited for the study. Blood samples (5.0ml) was collected from each subject for the analysis of the parameters using the Mindray 530 BC automated analyzer, Mindray, Japan. The data was analyzed using T-test with level of significance set at p < 0.05. The result revealed significant increase in the differential leukocyte ratios involving the Monocyte Lymphocyte Ratio (MLR) (0.38 ± 0.15 ±0.017) and the Neutrophil Lymphocyte Ratio (NLR) (3.15 ± 3.57 vs 1.65 ± 0.65) in the type 2 diabetic patients and the non-diabetic controls. This finding demonstrates alterations in differential leukocyte ratios which supports occurrence of parainflammation in type 2 diabetic patients.

Keywords: type 2 diabetes, differential leukocyte ratios, parainflammation

Introduction

Diabetes mellitus is a group of metabolic disorders characterized by abnormal carbohydrate metabolism resulting in chronic hyperglycemia caused by defective insulin production, action or both (1,2). Type 2 Diabetes Mellitus (T2DM) is the most prevalent type of diabetes and accounts for about 90-95 of diabetes cases (3-5). It’s global prevalence has increased from 4.7% (108 million) in 1980 to 9.3% (463 million) in 2019 and is postulated to increase to 10.2% (578 million) by 2030 as well as 10.2% (578 million) in 2030 (6,7). It is also estimated that 15.5% (9.8-27.8 million) people have type 2 diabetic mellitus with Nigeria having the highest burden of cases(8). Parainflammation otherwise known as low grade chronic inflammation is a shift in the inflammatory response from short to long-lived subclinical inflammation that causes breakdown of the immune tolerance (9,10). It involves a persistent harmful degenerative process in which neutrophils, macrophages, lymphocytes and plasma cells are released in the tissues producing antibodies, cytokines, growth factors, and enzymes hence contributing to the progression of tissue damage, fibrosis, granuloma, and/or metabolic disturbances(11,12). Peripheral blood differential leukocyte subsets has been considered as important markers of parainflammation and alterations in their ratios has been implicated in chronic diseases such as diabetes mellitus type 2 (13). There is currently a paucity of data on the differential leukocyte ratios in patients with type 2 diabetic mellitus in the Enugu State University of Science and Technology Teaching Hospital, Enugu State, Nigeria. The present study was therefore designed to determine the differential leukocyte ratios in type 2 diabetic patients compared to non-diabetic controls.

Materials and Methods

Study Area

The study was conducted in the Enugu State University of Science and Technology Teaching Hospital, Parklane, Enugu State, Nigeria. The State derived its name from its capital and largest city, Enugu. It has an area of 7,161km² with a population of 3,267,837 comprising mainly the Igbo tribe of the South Eastern Nigeria. It lies between longitudes 6°30’E and 6°55’E and latitudes 5°15’N and 7°15’E. It consists of three senatorial divisions namely Enugu East, Enugu North and Enugu West(14). The teaching hospital is the major tertiary health facility for the State and is located at the centre of the Enugu metropolis (Parklane) for easy accessibility to residents.
Subject Recruitment

Subject selection was based on a simple random sampling procedure from a population of diabetic patients who gave their consent and has met the inclusion criteria.

Inclusion Criteria

1. Patients already diagnosed with type 2 diabetes mellitus.
2. Non-diabetic individuals without known coronary artery disease, cerebrovascular disease, peripheral vascular disease or any systemic disease.
3. Age between 20-25 years.
4. Gender of both males and females.

Exclusion Criteria

1. Males with hemoglobin below 13g/dl and females with hemoglobin below 12g/dl.
2. Subjects with abnormal platelet count.
3. Non-diabetics with coronary artery disease, cerebrovascular, peripheral vascular disease, systemic diseases and diabetics on antiplatelet drugs such as aspirin or clopidogrel.
4. Subjects diagnosed with any form of tumor or malignancy.

Blood Sample Collection

Blood was collected from subjects using venipuncture(17). Subjects were made comfortable in a sitting position. A tourniquet was gently applied 2.5cm just above the antecubital fossa. The antecubital fossa was cleaned using a 70% alcohol in cotton wool. A hypodermic syringe and 21G needle was inserted into the lumen of the antecubital vein and five milliters (5ml) of blood was drawn quickly by a non-traumatic pulling of the syringe piston. This was dispensed into an EDTA bottle which was gently mixed.

Determination of the Differential Leukocyte Ratio

The differential leukocyte ratios involving the NLR, MLR,ELR and BLR were calculated manually from the values of the neutrophil, monocyte, Eosinophils and lymphocytes obtained from the full blood count results. NLR = Ratio of the neutrophil to the lymphocytes, MLR = Ratio of the monocyte to the lymphocytes, ELR = Ratio of the Eosinophil to the Lymphocytes and BLR = Ratio of the Basophil to Lymphocytes(15). The absolute count of the leukocyte subset was calculated as the product of its respective differential percentage and total leukocyte count.

Data Analysis

Data was analyzed using SPSS version 23 (SPSS Inc. Chicago). Statistical significance was defined as p < 0.05. Continuous variables (differential leukocyte ratios) were reported as means ± standard deviation (SD) from the mean. Differences in the continuous variables between the type 2 diabetes patients and the non-diabetic controls were determined by independent sample t-test.

Study Design

This is a cross-sectional case-controlled survey in which patients with type 2 diabetes mellitus served as the cases while age-matched healthy non-diabetics served as the controls.

Ethical Considerations

Ethical clearance was obtained from the Ethical Review Committee of the ESUT Teaching Hospital (ESUT NP/C-MAC/RA/034/vol. 1/290) as well as informed consent from the patients.

Sample Size

The sample size for the study was calculated using the Leslie Kish formula(15)

\[
 n = \frac{Z^2 PQ}{D^2}
\]

Where

- \( n \) = minimum required sample size
- \( Z \) = the α level of the coefficient interval or the standard normal deviate set at 1.96 corresponding to the 95% confidence interval.
- \( P \) = the proportion of the target population estimated to have diabetes mellitus 8.0%(16)
- \( D \) = the width of the confidence interval set at 0.05
- \( Q \) = (1-\( P \)); the proportion of non-occurrence.

Substituting into the formula

\[
 n = \frac{1.96 \times 1.96 \times 0.08(1-0.08)}{(0.05)^2} = 120
\]

Because the population of study is less than 10,000 the formula below was incorporated to calculate the actual sample size.

\[
 nf = \frac{n}{1+n/N}
\]

Where

- \( nf \) = desired sample size when population is < 10,000
- \( n \) = desired sample size when population is > 10,000
- \( N \) = estimate of the population size

Substituting

\[
 nf = \frac{120}{1+120/250} = 81.08
\]

For the purpose of non-compliance which may arise on the course of subject recruitment, 10% of the \( nf \) was added. 10% of 81.08 is 8.108. Therefore, the sample size for the study is 81.08 + 8.108 = 89.188, this was approximated to a minimum sample size of 89 subjects.

Results

The values of the differential leukocyte ratios revealed a significant increase in the NLR and MLR but a significant increase in the ELR and BLR in the type 2 diabetic patients compared to the controls (Table 1.). There was a significant increase in the NLR of female T2DM patients compared to the controls (Table 1.) There was a significant increase in the ELR and BLR in the type 2 diabetic patients compared to the controls (Table 1.) There was a significant increase in the ELR and BLR in the type 2 diabetic patients compared to the controls (Table 1.)
significant differences between the male and female cases as well as the male and female controls (Table 3).

Table 1: Mean values of differential leukocyte ratios in type 2 diabetic patients and controls

| Parameters | Reference Range | Type 2 diabetes (n = 120) | Controls (n = 120) | T-test (p-value) |
|------------|----------------|---------------------------|-------------------|-----------------|
| NLR        | 1.2-4.4        | 3.15 ± 3.57               | 1.65 ± 0.06       | 0.045*          |
| MLR        | 0.39-0.58      | 0.382 ± 0.86              | 0.159 ± 0.017     | 0.040*          |
| BLR        | 0.01-0.05      | 0.021 ± 0.44              | 0.020 ± 0.37      | 0.646           |
| ELR        | 0.03-0.07      | 0.05 ± 0.71               | 0.053 ± 0.52      | 0.810           |
| FBS (mmol/L) | 3.6-5.6    | 9.6 ± 1.21                | 3.6 ± 0.35        | 0.021*          |
| HbAIC (%)  | <7            | 9.54 ± 2.02               | 3.86 ± 1.12       | 0.007*          |

Key: NLR = Neutrophil lymphocyte ratio, MLR = monocyte lymphocyte ratio, BLR = Basophil lymphocyte ratio, ELR = eosinophil lymphocyte ratio, FBS = fasting blood sugar, HbAIC = glycated hemoglobin, *significant at p<0.05; Data expressed as Mean ±SD

Table 2: Differential Leukocyte ratio of type 2 diabetic patients and control based on gender

| Parameters | Male (test) | Female (test) | Male (control) | Female (control) | P-value |
|------------|-------------|---------------|----------------|------------------|---------|
| NLR        | 2.31 ± 0.77 | 3.47 ± 4.14   | 1.64 ± 058     | 1.65 ± 0.67      | 0.186*  |
| MLR        | 0.165 ± 0.165 | 0.381 ± 0.91 | 0.381 ± 0.91   | 0.365 ± 0.92     | 0.001   |
| BLR        | 0.16 ± 0.24  | 0.019 ± 0.79  | 0.02 ± 0.18    | 0.020 ± 0.43     | 0.03    |
| ELR        | 0.043 ± 1.11 | 0.040 ± 0.37  | 0.036 ± 0.90   | 0.041 ± 0.71     | 0.002   |

Key: NLR = Neutrophil lymphocyte ratio, MLR = monocyte lymphocyte ratio, BLR = Basophil lymphocyte ratio, ELR = Eosinophil lymphocyte ratio

Table 3: Posthoc Analysis of the platelet indices of Type 2 diabetic patients and control

| Group      | Parameters | NLR | MLR | ELR | ELR |
|------------|------------|-----|-----|-----|-----|
| T2DM       | Male vs T2D female | 0.229 | 0.616 | 0.010 | 0.021 |
| T2DM       | Male vs control (male) | 0.605 | 0.000 | 0.016 | 0.010 |
| T2DM       | Male vs control (female) | 0.594 | 0.000 | 0.018 | 0.010 |
| T2DM       | Male vs control (male) | 0.110 | 0.016 | 0.014 | 0.010 |
| T2DM       | Female vs control (female) | 0.087 | 0.010 | 0.010 | 0.010 |
| Male       | Controls vs female control | 0.930 | 0.461 | 0.989 | 0.055 |

Key: NLR = Neutrophil lymphocyte ratio, MLR = monocyte lymphocyte ratio, BLR = Basophil lymphocyte ratio, ELR = Eosinophil lymphocyte ratio

Discussion

The immune response to hyperglycemia is characterized by changes in the differential leukocytes involving the neutrophils, monocytes, basophils, eosinophils, and lymphocytes. Accordingly, changes in the neutrophil lymphocyte ratio (NLR), monocyte lymphocyte ratio (MLR), eosinophil lymphocyte ratio (ELR) and the basophil lymphocyte ratio (BLR) has been identified as an efficient marker of subclinical systemic inflammation in various diseases(18,19). A high MLR occurs when the monocyte count becomes high while the lymphocyte count becomes low, a high NLR occurs when the neutrophil count becomes high while the lymphocyte count becomes low, a high ELR occurs when the eosinophil count becomes high with the lymphocyte count low while a high BLR occurs when the basophil count becomes high with lymphocyte count low. Generally, high neutrophils, basophils, eosinophils and monocytes results in the secretion of superoxide radicals, cytokines and a variety of proteolytic enzymes by these cells which favors inflammatory process.

On the other hand, lymphocytes excerts a modulatory effect on the immune response to hyperglycemia with lymphocytopenia occurring as a result of increased apoptosis in lymphocytes thereby promoting a state of subclinical inflammation(20). The high NLR and MLR recorded in the present study is in agreement with the findings of Wang et al(21) and Bilgim et al(22) who recorded significant increase in the NLR and MLR in the type 2 diabetic patients compared to the non-diabetic controls. Noursy et al(23), Mertoglu and Gunay(24) reported significant increase in only the NLR while Mendes et al(25) showed higher neutrophil counts in patients.
with hyperglycemia compared to their normoglycemic counterparts but they did not find a difference in the NLR between the examined groups. The common similarity in the findings of these studies are increased neutrophil lymphocyte ratio which depicts a subclinical inflammation and a possible mechanism for this could be interruption of insulin signaling by inflammatory molecules which are released by the activated differential leukocytes. A limitation of the present study is its cross-sectional design which mitigated our ability to infer a casual relation between the differential leukocyte ratios and prognosis of the patients. It would be useful to measure if the values of the differential leukocyte ratios obtained are stable overtime. Also the existence of unrecognized confounding variables is could have occurred during this study. This is because some asymptomatic infections such as chronic infections with chlamydia pneumonia and Helicobacter pylori could affect the differential leukocyte ratios.

**Conclusion**

The findings of the present study showed that the Neutrophil to lymphocyte ratio and monocyte-to-lymphocyte ratio are inexpensive, and easily accessible parameters that could be applied for diagnosis and prognosis of type 2 diabetes mellitus.

**References**

1. Adane T, Arise F, Getaneh Z, Getawa T (2021). White blood cells and platelet profile of diabetic patients at University of Gondar Specialised Referral Hospital: A comparative cross-sectional study. Journal of Clinical Laboratory Analysis 35(e23808):1-7.
2. Arkew M, Yeman T, Mengistu Y, Gemechu K, Tesfaye G (2021). Hematological parameters of Type 2 Diabetic adult patient at Debre Berhan Referral Hospital, Northeast Ethiopia: A comparative cross-sectional study. Public Library of Science One 16(6): e0253286.
3. Narjis M, Noreen M, Safi SZ, Ilahi NE, Abmar SY, Alkhrurji AF (2021). Cross talk between complete blood count and progression of type II diabetes mellitus. Journal of King Saud University of Science 33:10149.
4. Ali MH, Hassan AJ (2019). Assessment of the alteration of blood indices in patients with type 2 diabetes mellitus: A cross-sectional study. Mustansiriya Medical Journal 18:24-29.
5. Kaur R, Kaur M, Singh J (2018). Endothelial dysfunction and platelet hyperactivity in type 2 diabetes mellitus: molecular insights and therapeutic strategies. Cardiovascular Diabetology 17(121):1-17.
6. Park JM, Lee HS, Park JY, Jung DH, Lee JW (2021). While blood cell counts as a predictor of incident type 2 diabetes mellitus among non obese adults: a longitudinal 10 year analysis of the Korean Genome and Epidemiology study. Journal of Inflammation Research 1(4):1235-1242.
7. Wang Y, Yang P, Yan Z, Liu Z, Ma Q, Zhang Z, Wang Y, Su Y (2021). The relationship between erythrocytes and diabetes mellitus. Journal of Diabetes Research 6656062:1-9.
8. Zimmermann M, Bumm C, Hazel N, Gray CM, Lwanda J (2018). Experiences of type 2 diabetes in Sub-Saharan Africa: a scoping review. Global Health and Research Policy 3:25.
9. Castro AM, Concha MLE, Melendez CAP (2017). Low grade inflammation and its relation to obesity and chronic degenerative diseases. Special Issues on Genetics 80:101-105.
10. Needham EJ, Helmy A. Menon DK (2019). The immunological response to traumati brain injury. Journal of Neuroimmunology 15:112-125.
11. Suguna S, Kusumadevi MS (2019). Correlation of HbA1C levels with monocyte-lymphocyte and platelet-lymphocyte ratios in type 2 diabetes of Bengaluru City. International Journal of Physiology 7(4):99-103.
12. Devamsh GN, Parvathi M, Madhumathi R, Raghavan L (2019). Study of neutrophil lymphocyte ratio in patients with type 2 diabetes mellitus and its correlation with glycemic control. International Journal of Advances in Medicine 6(5):1637-1641.
13. Chen W, Wang J, Ye B, Zhou J, Wang W (2021). The population characteristics of the main leukocyte subsets and their association with chronic diseases in a community-dwelling population: a cross-sectional study. Primary Health Care Research and Development 22(e18):1-8.
14. Ndulue DC, Ayadiuno RU, Mozie AT, Ogbu CT (2021). Spatial variation in the level of awareness and application of climate change polices and laws in Enugu State, South East Nigeria. Psychology and Education 58(2): 6466-6477.
15. Akpan UO, Bassey IE, Nwatu NN, Ofor CJ (2018). A comparative study on ABO blood group and fertility hormones in infertile women in Calabar, Southern Nigeria. Annals of Medical Physiology 2(2): 19-24.
16. Cookev SN, Gomb VE, Wariboko CM (2022). Prevalence of diabetes in rural communities in South South and South East Nigeria, a retrospective cross-sectional community based survey. IOSR Journal of Dental and Medical Sciences 21(2):26-32.
17. Shonde-Adebola KB, Shokunbi WA, adebola MB (2021). Prevalence of Von Willebrand Disease among Nigerian youths in Ibadan, South-Western Nigeria. Open access Library Journal 8(e7789):1-8.
18. Moosmann J, Krusemark A, Dittrich S, Amar T, Rauch M, Woelfle J, Metzler M, Zierk J (2022). Age-and-sex-specific pediatric reference intervals for neutrophil-to-lymphocyte ratio; lymphocyte-to-monocyte ratio, and platelet-to-lymphocyte ratio. International Journal of Laboratory Hematology 44:296-301.
19. Cekici Y, Yilmaz M, Secen O (2019). New inflammatory indicators: Association of high eosinophil-to-lymphocyte ratio and low lymphocyte-to-monocyte ratio with smoking. Journal of International Medical Research 49(9):4292-4303.
20. Pezhman L, Tahrani A, Chimen M (2021). Dysregulation of leukocyte trafficking in type 2 diabetes; mechanisms and potential therapeutic avenues. Frontiers in Cell and Development Biology 9(624184):1-19.

21. Wang J, Zhu Q-W, Cheng X-Y, Sha C-Y, Cui Y-B (2020). Clinical significance of neutrophil-lymphocyte ratio and monocyte-lymphocyte ratio in women with hyperglycemia. Postgraduate Medicine 132(8):702-708.

22. Bilgims S, Aktas G, Kocak MZ, Atak BM, Kurtkulagi O, Duman TD, Savli H (2020). Association between novel inflammatory markers derived from hemogram indices and metabolic parameters in type 2 diabetic men. The Aging Male 23(5):923-927.

23. Moursy EY, Megalla MH, Mouftah RF, Ahmed SM (2015). Relationship between neutrophil-lymphocyte ratio and microvascular complications in Egyptian patients with type 2 diabetes. American Journal of Internal Medicine 3(6):250-255.

24. Mertoglu C, Gunay M (2017). Neutrophil-lymphocyte ratio and platelet-lymphocyte ratio as useful predictive markers of prediabetes and diabetes mellitus. Diabetes Mellitus Syndrome 1(1):127-131.

25. Mendes BB< Oliveira ACR, Alcantara KC (2019). Comparison of the neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in normoglycemic and hyperglycemic subjects. Einstein (Sao Paulo) 17:eAo4403.

Copyright (c) 2021 The copyright to the submitted manuscript is held by the Author, who grants the Clinical Medicine and Health Research Journal a nonexclusive license to use, reproduce, and distribute the work, including for commercial purposes.

This work is licensed under a Creative Commons Attribution 4.0 International License.