Clinical Medicine and Health Research Journal (CMHRJ)
Volume 02, Issue 05, September - October, 2022
Page No. 240-244

Research Article

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

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Received: 20 September, 2022 Accepted: 20 October, 2022 Published: 26 October 2022

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 $\pm$ vs 0.15 $\pm$0.017) and the Neutrophil Lymphocyte Ratio (NLR) (3.15 $\pm$ 3.57 vs 1.65 $\pm$ 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 10.9% (700 million) by 2045(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 7,161km^2 with a population of 3,267,837 comprising mainly the Igbo tribe of the South Eastern Nigeria. It lies between longitudes 6^{o}30'E and 6^{o}55'E and latitudes 5^{o}15'N and 7^{o}15^{o}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 $\pm$ 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)

$$n = \frac{Z^{\alpha 2} PQ}{D^2}$$

Where n = minimum required sample size

2α = 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 in 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 nt = desired sample size when population is < 10,000

n = desired sample size when population is > 10,000 = 120

N = estimate of the population size = 250

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 male controls (Table 2) while the posthoc analysis confirmed significant differences observed in the differential leukocyte ratios of the type 2 diabetic patients and controls and no

significant differences between the male and female cases as well as the male and female controls (Table3).

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 ± 71	0.053 ± 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 Meant ±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 rate, ELR = Eosinophil lymphocyte ratio.

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

Group		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 monocyutes 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.

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