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ORIGINAL ARTICLE

Association between Haemoglobin Patterns, Platelet Lymphocyte Ratio, and Neutrophil Lymphocyte Ratio in Diabetic and Non-Diabetic Individuals in Port Harcourt, Nigeria

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ABSTRACT

Introduction: Due to elevated blood sugar, diabetes mellitus is the most common metabolic disease with diverse etiologies. The purpose of this study is to assess the relationship between diabetics' haemoglobin electrophoretic pattern, platelet lymphocyte ratio (PLR), and neutrophil lymphocyte ratio (NLR). This cross-sectional study was conducted on a total of 100 participants, 50 of whom had been diagnosed as having diabetes and the remaining 50 were non-diabetic patients who were chosen from a consenting population at a private hospital in Port Harcourt, Rivers State.

Materials and Methods: The subjects were 50 years of age and older. The whole blood count 3-part analyzer was used to examine the blood samples to obtain the PLR and NLR values, and an electrophoresis machine was employed to ascertain the participants' haemoglobin electrophoretic pattern.

Results: Thirty-five (35) of 100 participants had the genotype AA, while 15 had the electrophoretic pattern AS. Regarding PLR value, there was no statistically significant difference between the diabetic group and the control group PLR value (p = 0. 0.4418) and NLR value (p = 0.7540) at 108.6 ± 40.4 102.7±36.3, respectively. No significant relationship occurred between Absolute Lymphocyte Count and Absolute Neutrophil Count (p = 0.7711) and (p =0.7815) at (2.41 ± 0.83) (2.46 ± 0.81) and (3.10 ± 1.73) (246.1 ± 86.4) for both study subjects and control subjects respectively. Platelet Count at 246.1 ± 86.4 and 242.4 ± 75.9 for both groups was also insignificant statistically (p =0.8246).

Conclusion: The study was unable to find a statistically significant difference between the groups. Further testing utilizing other haematological indications would be required, in addition to considering the stage of diabetes, family history, environment, and way of life.

Keywords: Evaluation, Hemoglobin Electrophoretic, Patterns, Platelet-Lymphocyte Ratio (PLR), Neutrophil-Lymphocyte ratio (NLR), Diabetic

INTRODUCTION

High blood sugar levels are a symptom of diabetes mellitus, which can be brought on by the pancreas' inability to make enough insulin or to use the insulin produced properly. Diabetes mellitus is a metabolic group [1]. Diabetes is characterized by chronic hyperglycemia, which causes alterations in the metabolism of proteins, lipids, and carbohydrates. A persistent rise in blood glucose levels characterizes chronic hyperglycemia. Polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased appetite) are all symptoms of hyperglycemia [2]. With over 170 million cases worldwide and 1.7% of the population in Nigeria impacted, diabetes mellitus is a serious issue in both Nigeria and the rest of the world [3]. In Rivers State, the prevalence of diabetes mellitus has risen to roughly 42.7%. However, according to the International Diabetes Federation (IDF), the rate of diabetes among persons aged 40 and older increased by around 9.3% in 2019 and is expected to reach about 10.9% by 2045 [4].

NLR (Neutrophil Lymphocyte Ratio) and PLR (Platelet Lymphocyte Ratio) have recently been found to have a predictive power in diabetes complications. Both are significant inflammatory markers. PLR and NLR are two novel biomarkers for systemic inflammation that can be easily collected through routine blood tests and used in therapeutic settings [5,6,7]. NLR has been largely researched as a biomarker that demonstrates the systemic inflammatory state compared to individual biomarkers. It is cost-effective and easy to detect [8,5]. As a ratio of neutrophils to lymphocytes, NLR provides greater accuracy than a single measurement and effectively reflects the systemic inflammatory response [9]. Reports show that hyperglycemia affects the number and function of circulating neutrophils [10]. According to a study by Moore-Igwe et al. [11], NLR is associated positively with inflammation.

Patients with impaired glucose tolerance may have an elevated risk of cardiovascular disease and an increased White Blood Cell Count (WBCC) due to numerous metabolic syndrome components [12]. Inflammation is commonly seen to play a vital role [13]. White blood cell (WBC) count and its subtypes, including neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), are viable innovative indicators of systemic inflammation in various diseases, in line with considerable proof.

It has been shown that a genotype score can predict a person's predisposal and that this prediction holds true even when common clinical risk factors are taken into account. Several studies have demonstrated a genetic advantage for people with haemoglobin AS (HBAS) and haemoglobin SS (HBSS) over those with HBAA for defense against Plasmodium falciparum [14]. However, prolonged hyperglycemia, the "hall mark" of diabetes mellitus, is the key component in the pathogenesis. There is currently no information on the potential role of the haemoglobin genotype trait in the prevention or susceptibility to the development of diabetes mellitus. Therefore, the initial goal of the current study was to evaluate the likelihood that haemoglobin genotype would be associated.

MATERIALS AND METHODS

Study Design

This cross-sectional study was conducted among patients who sought treatment at a private hospital.

Study Area

At Port Harcourt, the state capital of Rivers State, this facility-based cross-sectional study was conducted in a private hospital. Port Harcourt has a population of 1,865,000 and a location along the Bonny River, it is a city in Nigeria's South-South geopolitical zone, usually referred to as the Niger Delta.

Study Population

100 male and female subjects, 50 years of age or older, in total, were employed in this study. The other 50 test participants were diabetic patients, while the other 50 served as controls and did not have diabetes mellitus.

Collection of Blood Samples, Storage and Transportation

According to Chesbrough's [15] guidelines, blood samples were drawn from the veins using a vacutainer tube and needle and placed in visibly labeled EDTA anticoagulated bottles. Using a triple packaging process, these samples were then quickly transported to the laboratory where they were kept at 4°C until testing.

MATERIALS AND METHODS

Determination of Full Blood Count

Method: Sysmex XE-2100 Haematology Automated Analyzer automation.

The roller mixer was used to mix all of the blood samples that had been collected in EDTA bottles. The laboratory numbers of each blood sample were then serially entered into the machine. The blood samples were also manually gently shaken before being aspirated using the sample probe (Orifice). At a cool temperature, the machine was turned on and allowed to boot and pick up. The equipment appeared to be ready for operation when a green light showed. A new sample was examined twice, the results were compared, and the first finding served as the day's control. The machine printed out the results, which were then recorded.

Determination of Genotype

Method:

A paper wick was wet and inserted along each divider of the electrophoresis tank, 50ml of this buffer was placed in each of the outer buffer compartments, the cellulose acetate membrane was soaked in the buffer for 5 minutes, and 5ul of the patient's blood was added. 20ul of the washed packed cells were added to 60ul of laying solution and gently mixed for 5 minutes after being rinsed twice in normal saline with about 40ul of packed red cells. Two (2) microscintillation detectors were used to determine whether the sample was present by placing the cellulose acetate plate in the electrophoresis tank with the cellulose acetate paper in contact with the anode and cathode wick. After being taken out of the working buffer, the cellulose acetate was blotted between two sheets of fresh blotting paper until it was almost dry. By comparing the migration distance with the known controls separated under the same conditions as the test sample at the end of 25 minutes, the haemoglobin types contained in the patient sample were identified. The results were read and written down.

Statistical Analysis

Simple percentage calculations were used to statistically analyze the acquired data. Tables were used to display the data.

RESULTS

Demographic Details of Study Participants

The study included 100 individuals in

total. Those with diabetes were 50, whereas subjects without diabetes were 50. Males without diabetes were 25, while those with diabetes were 27. Females without diabetes were 28, while those with diabetes were 23. The number of diabetic participants with the AA haemoglobin genotype was 35, while the number of diabetic subjects with the AS genotype was 15. Table 1 provides specifics.

Table 1: Demographic Details of Study Participants

Parameters	**Number**
Total Number of Subjects	100
Total Number of Diabetic Subjects	50
Total Number of Non-Diabetic Subjects	50
Total Number of Diabetic Males	27
Total Number of Diabetic Females	23
Total Number of Diabetics with AA Genotype	35
Total Number of Diabetics with AS Genotype	15

Comparison of Studied Parameters in Diabetic and Non-diabetic Subjects

When the PLR, NLR, absolute neutrophil count, absolute lymphocyte count, and platelet count were examined between diabetes and non-diabetic participants, there was no statistically significant (p 0.05) difference found. Table 2 provides specifics.

Comparison of Studied Parameters in Diabetic Subjects based on Haemoglobin Genotype

Those with diabetes and those without the disease showed no statistically significant (p0.05) difference. No statistically significant (p0.05) difference was discovered when the PLR, NLR, absolute lymphocyte count, absolute neutrophil count, and platelet count were evaluated among diabetic subjects based on changes in haemoglobin genotype. Details are provided in Table 4.5. When the PLR, NLR, platelet, absolute neutrophil, absolute lymphocyte, and all of these numbers were compared. Details are provided in Table 2.

Table 2: Comparison of Studied Parameters in Diabetic and Non-Diabetic Subjects

Parameters	**Diabetic** **Mean ± SD**	**Non-Diabetic** **Mean ± SD**	**p-value**
PLR	108.6 ± 40.4	102.7 ± 36.3	0.4418 (NS)
NLR	1.33 ± 0.65	1.37 ± 0.68	0.7540 (NS)
Absolute Lymphocyte Count	2.41 ± 0.83	2.46 ± 0.81	0.7711 (NS)
Absolute Neutrophil Count	3.10 ± 1.73	3.20 ± 1.71	0.7815 (NS)
Platelet Count	246.1 ± 86.4	242.4 ± 75.9	0.8246 (NS)

Based on the electrophoretic pattern genotype, a comparison of studied parameters in diabetic subjects was performed.

Table 3: Comparison of Studied Parameters in Diabetic Subjects based on Electrophoretic Pattern Genotype

Parameters	**Diabetic AA** **Mean ± SD**	**Non Diabetic AS** **Mean ± SD**	**p-value**
PLR	108.5 ± 40.1	102.4 ± 39.1	0.6191 (NS)
NLR	1.33 ± 0.68	1.34 ± 0.59	0.9818 (NS)
Absolute Lymphocyte Count unit	2.36 ± 0.79	2.58 ± 0.92	0.3914 (NS)
Absolute Neutrophil Count unit	2.91 ± 1,32	3.55 ± 2.42	0.2424 (NS)
Platelet Count	242.7 ± 85.6	251.2 ± 86.2	0.7465 (NS)

When the PLR, NLR, absolute lymphocyte count, absolute neutrophil count, and platelet count were examined amongst diabetic participants based on variations in haemoglobin genotype, no statistically significant (p0.05) difference was found. Table 3 provides specifics.

DISCUSSION

The demographic and laboratory findings of the healthy group and the diabetes participants were compared in the study (comprising of 50 diabetic subjects and 50 non-diabetic subjects). Although the PLR value in the diabetes group was higher than in the healthy group, there was no statistically significant difference.

The study revealed that the PLR, NLR, Absolute Lymphocyte Count, Absolute Neutrophil Count and Platelet Count were statistically insignificant respectively. This outcome is consistent with a study by Dik [16], who found out that the PLR and NLR on the diabetic group was insignificant. However, a study by Onalan and Gokalp [17] on 100 diabetic patients and 100 healthy controls indicated that the NLR and PLR values in the diabetic group were greater than those in the healthy group, and this difference was statistically significant.

The study is in disparity with a study carried out by Hai et al. [18] whose result showed that higher NLR levels were related to elevated poor glycemic control in T2DM patients and NLR levels in T2DM patients were significantly higher than those in non-T2DM patients.

Atak et al. [19] reported that the median PLR in patients with type 2 diabetes was significantly elevated compared to healthy controls, suggesting that PLR could be a useful marker for predicting the onset and management of type 2 diabetes.

Also, Ahmed et al. [20] found that patients with poor glycemic management had higher NLR and PLR values, but the difference was not statistically significant which is in tandem with our study.

Generally, the study suggests a lack of marked variation in these hematological parameters between both groups being studied, mean absolute lymphocyte counts, absolute neutrophil counts and mean platelet counts, which were all statistically insignificant respectively. This finding may indicate that, in the absence of specific complications such as infections or other comorbidities, the inflammatory response in diabetes patients does not significantly impact these particular parameters. There is a strong discrepancy in our study with the findings of Wu and Gao [21], whose research showed the lymphocyte count in the diabetes with COVID-19 group

as decreased when compared with the non -diabetic group.

This difference in findings may be attributable to several factors, including the presence of a secondary inflammatory stimulus (i.e., COVID-19) which likely amplified the immune response and caused measurable changes in lymphocyte counts.

In the context of diabetes alone, it is possible that the chronic low-grade inflammation typically associated with the condition does not reach a threshold sufficient to significantly alter these hematological parameters, as seen in our study. Additionally, the discrepancy may reflect differences in the study populations, methodologies, or clinical settings. Conversely, our study focuses solely on diabetes patients without any acute inflammatory or infectious conditions, which may account for the lack of significant changes in lymphocyte, neutrophil, and platelet counts.

Another possible explanation is that our findings may reflect effective glycemic control or the absence of advanced diabetes-related complications in the studied population. Poor glycemic control and advanced diabetes are associated with greater immune dysfunction and inflammation, which may manifest as altered hematological profiles.

The PLR, NLR, Absolute Lymphocyte Count unit, Absolute Neutrophil Count unit and Platelet Count showed statistically significant value when the diabetic group was compared to non-diabetic group. This finding suggests that diabetes may not significantly suppress these haematological parameters in AA individuals, and the AS genotype in non-diabetics does not appear to confer a distinct immunological profile in terms of lymphocyte levels. Bailin et al. [22] found that T lymphocyte counts are lower in diabetic patients than in non-diabetic patients. Although Akpan & Okon's study showed that haemoglobin-AS offers protection against diabetes mellitus, he also discovered that there were less AS genotype participants with the condition than in the control group.

The mean absolute neutrophil count was higher in non-diabetic AS individuals compared to diabetic AA individuals but the difference was not statistically significant. Although neutrophils are typically elevated in systemic inflammatory states, the non-significance may indicate that diabetes does not induce a strong neutrophilic response in the AA genotype, and the AS genotype in non-diabetic individuals does not result in a substantially different neutrophil count. A statistically significant difference in absolute count of neutrophil was seen in T2DM patients compared to control in a research carried out by Mesay et al. [23].

The mean platelet count was slightly lower in diabetic AA individuals compared to non-diabetic AS individuals, but this difference was also not statistically significant. Platelet count is an important marker of coagulation and inflammation, and its lack of variation suggests that diabetes and the AA genotype do not significantly alter platelet levels, nor does the AS genotype in non-diabetic individuals. The results of Mesay et al. [23] also showed elevated platelet count in study subjects as compared to non-diabetic subjects.

The lack of statistically significant differences across all studied parameters suggests that diabetes (in AA individuals) and the AS genotype (in non-diabetics) may not independently or substantially impact the hematological and inflammatory profiles measured by PLR, NLR, absolute lymphocyte count, absolute neutrophil count, and platelet count.

These findings are consistent with the hypothesis that neither diabetes (in individuals with normal hemoglobin genotype) nor the AS genotype independently triggers significant systemic inflammation or hematological alterations in the studied population.

The results also indicate that factors beyond

genotype or diabetes status, such as glycemic control, duration of diabetes, or comorbidities, may play a more critical role in driving inflammation and hematological changes. A study by Moore – Igwe and Chukwu [24] on C-reactive protein in elderly diabetes mellitus patients revealed C-reactive protein (CRP) levels in diabetic patients were significant, statistically higher than non-diabetic controls. Hence, investigating other inflammatory and coagulation markers, such as C-reactive protein (CRP) or fibrinogen levels, may have provided additional insights into the inflammatory status of these populations.

Limitations and Recommendations

Further studies should consider including diabetic individuals with the AS genotype to evaluate potential interactions between diabetes and hemoglobinopathy on inflammatory and hematological markers. In a bid to explore the interplay between diabetes, genotype, and inflammation, there is need to carry out another research. Moreso, it is imperative to also further conduct an investigation into research with larger, more diverse populations to deepen understanding of these relationships.

CONCLUSION

This study underlines the significance of clinical context, comorbidities, and glycemic control in interpreting hematological markers in diabetic patients. It highlights that factor like disease progression and glycemic management may have a greater influence on these parameters than genotype or diabetes status alone.

CONSENT AND ETHICAL APPROVAL

Informed consent was obtained from apparently healthy subjects prior to enrolment upon approval by the Department of Medical Laboratory Science, Rivers State University, Port Harcourt.

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