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Significance of Some Markers of Systemic Inflammation and Platelet Activation in Patients with Type 2 Diabetes Mellitus in the Enugu State University of Science and Technology Teaching Hospital Enugu State Nigeria

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Abstract

Diabetes mellitus has over the years become a public health challenge and a complex disease which is characterized by chronic hyperglycemia that results in microvascular and macro vascular complications. The present study was designed to determine (i) The concentration of markers of platelet activation and (ii) The concentration of markers of systemic inflammation in patients with type 2 diabetes mellitus in Enugu State University of Science and Technology Teaching Hospital. A total of 240 subjects comprising 120 Type 2 Diabetic Mellitus (T2DM) (60 males and 60 females) aged 20-55 years and 120 apparently healthy age and gender-matched controls were recruited for the study. Blood samples (5.0 ml) were collected from each subject for the analysis of the parameters using Mindray 530 BC automated analyzer, Mindray Japan. The data was analysed using T-test and level of significance set at $p < 0.05$. The result revealed significant increase in the markers of platelet activation involving the Mean Platelet Volume (MPV) (12.17 ± 0.78 vs. 7.80 ± 1.18) Platelet Distribution Width(PDW) (14.41 ± 1.15 vs. 9.30 ± 0.92), Plateletcrit (PCT) (0.27 ± 0.32 vs. 0.17 ± 0.00) and markers of systemic inflammation involving the Neutrophil Lymphocyte Ratio(NLR) (3.15 ± 3.57 vs. 1.65 ± 0.65), Platelet Lymphocyte Ratio (PLR) (100.99 ± 8.50 vs. 83.88 ± 3.99) and the Monocyte Lymphocyte Ratio (MLR) (0.382 ± 0.86 vs. 0.15 ± 0.017) in the T2DM and controls. This finding demonstrates alterations in the markers of platelet activation and systemic inflammation in T2DM patients.

Keywords

T2DM, Platelet activation, Systemic inflammation, Enugu

Introduction

Diabetes mellitus is a group of metabolic disorders characterized by abnormal carbohydrate metabolism resulting in chronic hyperglycemia caused by detective 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 diabetes mellitus in sub-Saharan Africa with Nigeria having the highest burden of cases [8]. There has been renewed interest on the Neutrophil Lymphocyte Ratio (NLR), Platelet Lymphocyte Ratio (PLR) and Monocyte Lymphocyte Ratio (MLR) as well as the Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Plateletcrit (PCT) as efficient markets of systemic inflammation and platelet activation underlying the development of diabetic complications [1]. Although documented in some populations, there is currently a paucity of scientific data on the markers of systemic inflammation and platelet activation in patients with T2DM accessing clinical care in the Enugu State University of Science and technology Teaching Hospital (ESUTH) Parklane, Enugu State.

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Materials and Methods

Study area

The study was conducted with blood samples from patients attending the diabetic clinic of the Enugu state University of Science and technology (ESUT) Teaching Hospital, Enugu State in the South East geopolitical zone of Nigeria. The state derived its name from her capital and largest city, Enugu. It has an area of 7,161 km^2 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 [9]. The ESUT 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 Enugu residents.

Study design

This is a cross-sectional case-controlled survey in which patients with type 2 diabetes mellitus serve as the case while age-matched healthy non-diabetic 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 [10].

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

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% [11]

D = The width of the confidence internal set at 0.05.

Q = (1-p); the proportion of non-occurrence substituting into the formula

$$n = \frac{1.96 \times 1.96 \times 0.08(1\text{-}0.08)}{(0.05)^2}$$

= 120

Subjects recruitment

Subject selection was based on a simple random sampling procedure from a population of diabetic patients who gave their consent to participate in the study.

Inclusion criteria

1. All consenting Type 2 Diabetic patients on treatment were chosen as cases.
2. All consenting non-diabetic healthy adults were chosen as controls.

Exclusion criteria

1. Nutritional anemia can be cause of reactive thrombocytosis, therefore male and female patients having mean hemoglobin (Hb) < 12 g/dL and < 11 g/dL respectively were excluded from the study.
2. Non diabetic individuals with any diagnosed malignancy, thrombocytopenia, thrombocytosis or systemic disease were excluded.

Blood sample collection

Blood was collected from subjects using venipuncture [12]. Subjects were made comfortable in a sitting position. A tourniquet was gently applied 2-5 cm 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 milliliters (5 ml) 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 markers of platelet activation

The novel markers of platelet activation involving the MPV, PDW and PCT were determined by performing an automated full blood count using Mindray 530 BC auto analyzer, Japan. The sample was aspirated by letting the machine sample probe into the blood sample and then pressing the probe button. Approximately 20 µl of blood was aspirated by the auto analyzer. The result of the MPV, PDW and PCT were displayed in the screen after about 30 seconds as part of the full blood count result. A printout copy of results is released on the thermal printing paper [13].

Determination of markers of systemic inflammation

The novel markers of systemic inflammation involving the NLR, PLR and MLR were calculated manually from the values of the neutrophil, monocyte platelet and lymphocytes obtained from the full blood count results. NLR = Ratio of the neutrophil to the lymphocytes, PLR = Ratio of the platelet count to the lymphocyte while MLR = Ratio of the monocyte to the lymphocytes [14].

Data analysis

Data was analysed using SPSS version 23 (SPSS Inc. Chicago). Statistical significance was defined as $p < 0.05$. Differences in the markers of platelet activation and systemic inflammation between the cases and controls was tested using t-test.

Results

The values of the markers of systemic inflammation revealed significantly increased NLR, PLR and MLR in the Type 2 diabetic patients compared to controls (Table 1). The values of the markers of platelet activation revealed significantly

increased MPV, PDW and PCT 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 Posthoc analysis confirmed significant differences in markers of systemic inflammation and platelet activation between the T2DM cases and controls and no significant differences between the male and female cases as well as the male and female controls (Table 3).

Discussion

The combination of PCT, PDW and MPV have been shown to be efficient marker of subclinical platelet activation in various diseases [15]. In the present study, we recorded significantly increased PCT, PDW and MPV in the patients with Type 2 diabetes mellitus compared to controls. This is similar to the findings of Alhadas, et al. [16] who also recorded significantly increased PCT, PDW and MPV in T2DM patients compared to controls. However, our present finding is not in agreement with the findings of Akinsegu, et al. [13], Swaminathan, et al. [17] and Tejeswini, et al. [18] who recorded only significant increases in MPV of T2DM patients compared to controls. Other studies by Karthikeyan, et al. [19], Shilpi and Potekar [20] and Jaben, et al. [21] recorded significant increases in the MPV and PDW while Gowthan [22] recorded significant increase in the MPV and PCT. The PCT is a measure of the total platelet mass, PDW is a measure of platelet size while the MPV is a measure of platelet activity. An increased PCT, PDW and MPV recorded in the present

Table 1: Mean values of markers of systemic inflammation and platelet activation of Type 2 diabetic mellitus cases and controls.

Parameters	Reference Range	type 2 Diabetes (n = 120)	Controls (n = 120)	T-test (p-value)
MPV (fl)	9.0-13.0	12.17 ± 0.78	7.80 ± 1.18	0.001
PDW (%)	10.0-18.0	14.41 ± 1.5	9.30± 0.92	0.004
PCT (%)	0.22-0.24	0.27 ± 0.32	0.17 ± 0.01	0.036
NLR	1.2-4.4	3.15 ± 3.57	1.65 ± 0.06	0.045
PLR	75-199	100.99 ± 8.50	83.88 ± 3.99	0.010
MLR	0.39-0.58	0.382 ± 0.86	0.159 ± 0.017	0.040
FBS (mmol/l)	3.6-5.6	9.6 ± 1.21	3.6 ± 0.35	0.021
HbA1C (%)	< 7	9.54 ± 2.02	3.86 ± 1.12	0.007

Key: MPV: Mean Platelet Volume; PDW: Platelet Distribution Width; PCT: Plateletcrit; NLR: Neutrophil Lymphocyte Ratio; PLR: Platelet Lymphocyte Ratio; MLR: Monocyte Lymphocyte Ratio; FBS: Fasting Blood Sugar; HbA1C: Glycated Hemoglobin

Table 2: Markers of coagulation activation and systemic inflammation of Type 2 diabetic patients in ESUTH based on gender.

Parameters	Male (test)	Female (test)	Male (control)	Female (control)	P-value
MPV	12.65 ± 0.089	11.98 ± 0.65	7.67 ± 1.22	7.91 ± 1.22	0.001
PDW	14.12 ± 1.52	14.38 ± 0.99	9.33 ± 1.0	927 ± 0.91	0.012
PCT	0.27 ± 0.37	0.25 ± 0.30	0.16 ± 0.05	0.16 ± 0.03	0.043
NLR	2.31 ± 0.77	3.47 ± 4.14	1.64 ± 0.58	1.65 ± 0.67	0.186*
PLR	82.81 ± 4.60	84.29 ± 3.71	94.7 ± 5.45	106.65 ± 6.09	0.016
MLR	0.165 ± 0.165	0.381 ± 091	0.381 ± 0.91	0.365 ± 0.92	0.001

Key: MPV: Mean Platelet Volume; PDW: Platelet Distribution Width; PCT: Plateletcrit; NLR: Neutrophil Lymphocyte Ratio; PLR: Platelet Lymphocyte Ratio; MLR: Monocyte Lymphocyte Ratio

Table 3: Posthoc Analysis of coagulation activation and systemic inflammation markers of Type 2 diabetic patients in ESUTH.

Group		MPV	PDW	PCT	NLR	PLR	MLR
T2DM	Male vs. T2DM Female	0.021	0.453	0.01	0.229	0.033	0.616
T2DM	Male vs. Control (male)	0.010	0.01	0.016	0.605	0.001	0.000
T2DM	Male vs. Control(Female)	0.010	0.001	0.018	0.594	0.001	0.000
T2DM	Female vs. Control(male)	0.010	0.001	0.014	0.110	0.046	0.016
T2DM	Female vs. Control(Female)	0.001	0.036	0.010	0.087	0.001	0.010
Male	Control vs. Female Control	0.055	0.903	0.989	0.938	0.010	0.461

Key: MPV: Mean Platelet Volume; PDW: Platelet Distribution Width; PCT: Plateletcrit; NLR: Neutrophil Lymphocyte Ratio; PLR: Platelet Lymphocyte Ratio; MLR: Monocyte Lymphocyte Ratio

study suggests platelet activation has been proposed as the mechanism underlying hypercoagulable state and resultant macrovascular complications in T2DM patients. This is because activated platelets become larger in size, synthesize more serotonin, β-thromboglobulin and thromboxane A_2 which aid faster aggregation of platelets and subsequent activation of the coagulation cascade.

The combination of the NLR, PLR and MLR has as well been shown to be an efficient marker of subclinical systemic inflammation in various diseases [23]. This study also recorded significantly increased PCT, MPV and PDW in the T2DM patients compared to the controls. This is not in agreement with the findings of Wang, et al. [24] and Bilgim, et al. [25] who recorded significant increase in the NLR and MLR, Mertoglu and Gunay [26] who recorded significant increase in the NLR and PLR as well as Chen, et al. [27] and Moursy, et al. [28] who recorded significant increase in only the NLR. A high PLR occurs when the platelet count becomes high or when the lymphocyte count becomes low, a high MLR, occurs when the monocyte count becomes high or the lymphocyte count becomes low while a high NLR occurs when the neutrophil count becomes high and the lymphocyte count low. Generally, high platelet counts reflects increased platelet activity and release of inflammatory cytokines, high neutrophil count reflects the secretion of superoxide radicals, cytokines and a variety proteolytic enzymes while high monocyte count leads to release of proinflammatory cytokines such as IL-12 and tumour necrosis factor which facilitates inflammatory processes. Lymphocytes exert a modulatory effect on the inflammatory response with lymphocytopenia occurring as a result of increased apoptosis in lymphocytes which could induce the expression of IL-10 and promote tissue repairmen.

Conclusion

The finding of the present study reveals increased markers of systemic inflammation and platelet activation in patients with type 2 diabetes mellitus. This suggests a major underlying factor to the development of microvascular and macrovascular complications in T2DM patients.

References

1. Adane T, Arise F, Getaneh Z, et al. (2021) White blood cells and platelet profile of diabetic patients at University of Gondar specialised referral hospital: A comparative cross-sectional study. J Clin Lab Anal 35: e23808.
2. Arkew M, Yemane T, Mengistu Y, et al. (2021) Hematological parameters of Type 2 diabetic adult patient at Debre Berhan Referral Hospital, Northeast Ethiopia: A comparative cross-sectional study. PLoS One 16: e0253286.
3. Narjis M, Noreen M, Safi SZ, et al. (2021) Cross talk between complete blood count and progression of Type II diabetes mellitus. J King Saud Univ Sci 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: 24-30.
5. Kaur R, Kaur M, Singh J (2018) Endothelial dysfunction and platelet hyperactivity in type 2 diabetes mellitus: Molecular insights and therapeutic strategies. Cardiovasc Diabetol 17: 1-17.
6. Park JM, Lee HS, Park JY, et al. (2021) White blood cell count 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. J Inflamm Res 1: 1235-1242.
7. Wang Y, Yang P, Yan Z, et al. (2021) The relationship between erythrocytes and diabetes mellitus. J Diabetes Res 2021: 6656062.
8. Zimmermann M, Bumm C, Hazel N, et al. (2018) Experiences of type 2 diabetes in Sub-Saharan African: A scoping review. Glob Health Res Policy 3: 25.
9. Ndulue DC, Ayadiuno RU, Mozie AT, et al. (2021) Spatial variation in the level of awareness and application of climate change policies and laws in Enugu State, South East Nigeria. Psychol Educ 58: 6466-6477.
10. Akpan UO, Bassey IE, Nwatu NN, et al. (2018) A comparative study on ABO blood group and fertility hormones in infertile woman in Calabar, Southern Nigeria. Ann Med Physiol 2: 19-24.
11. Cookey SN, Gomba VE, Wariboko CM (2022) Prevalence of diabetes in rural communities in South South and South East Nigeria, A retrospective cross sectional community based survey. J Med Dent Sci 21: 26-32.
12. 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.
13. Akinsegun A, Olusola DA, Sarah JO, et al. (2014) Mean platelet volume and platelet counts in type 2 diabetes mellitus on treatment and non-diabetic mellitus controls in Lagos, Nigeria. Pan Afr Med J 18: 1-5.
14. Klisic A, Kotur Stevuljevic J, Ninic A (2022) White blood cell count, its subsets and their indexes in type 2 diabetes mellitus. Arch Pharm 72: 193-199.
15. Inanir M, Sincer I, Gunes Y (2019) Platelet indices in type 1 diabetes mellitus. Exp Biomed Res 2: 69-75.
16. Alhadas HR, Santos SN, Freitas MMS, et al. (2016) Are platelet indices useful in the evaluation of type 2 diabetes patients. J Brasillian Pathol Med Lab S2: 96-102.
17. Swaminathan A, Amitkumar K, Gonapathy S, et al. (2017) Evaluation of mean platelet volume and other platelet parameters in subjects with type 2 diabetes mellitus. National J Physiol Pharm Pharmacol 7: 1-4.
18. Tejeswini V, Premalatha P, Krishnamuchuryulu PAV (2016) Role of mean platelet volume in individuals with type 11 diabetes mellitus. J Clin Pathol Forensic Med 7: 1-6.
19. Karthikeyan TM, Abinaya SA, Shiva PR, et al. (2021) A study of platelet indices in type 2 diabetes mellitus patients. Int J Recent Sci Res 12: 40868-40871.
20. Shilpi K, Potekar RM (2018) A study of platelet indices in type 2 diabetes mellitus patients. Indian J Hematol Blood Transfus 34: 115-120.
21. Jaben F, Rizvi H, Aziz F, et al. (2013) Hyperglycemic induced variations in hematological indices in type 2 diabetes. Int J Curr Adv Res 1: 322-334.
22. Gowtham A (2019) A study on platelet indices in type 11 diabetes mellitus. Indian J Hematol Blood Transfus 2: 10-13.
23. Moosmann J, Krusemark A, Dittrich S, et al. (2022) Age-and-sex-specific pediatric reference intervals for neutrophil-to-

lymphocyte ratio, lymphocyte-to-monocyte ratio and platelet-to-lymphocyte ratio. Int J Lab Hematol 44: 296-301.

24. Wang J, Zhu QW, Cheng XY, et al. (2020) Clinical significance of neutrophil-lymphocyte ratio and monocyte-lymphocyte ratio in women with hyperglycemia. Postgrad Med 132: 702-708.
25. Bilgins S, Aktas G, Kocak MZ, et al. (2020) Association between novel inflammatory markers derived from hemogram indices and metabolic parameters in type 2 diabetic men. Aging Male 23: 923-927.
26. Mertoglu C, Gunay M (2017) Neutrophil-lymphocyte ratio and platelet-lymphocyte ratio as useful predictive markers of prediabetes and diabetes mellitus. Diabetes Metab Syndr 1: 127-131.
27. Chen W, Chen K, Xu Z, et al. (2021) Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio predict mortality in patients with diabetic ulcer undergoing amputation. Diabetes Metab Syndr Obes 14: 821-829.
28. Moursy EY, Megalla MH, Mouftah RF, et al. (2015) Relationship between neutrophil-lymphocyte ratio and microvqascular complications in Egyptian patients with type 2 diabetes. Am J Intern Med 3: 250-255.

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