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Haematological parameters and their correlation with lipid profile in type 2 diabetes mellitus patients.

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Abstract
Background*: Diabetes mellitus is a chronic metabolic disease with a high prevalence rate worldwide. It is associated with various haematological and lipid abnormalities that lead to morbidity and mortality. The aim of this study was to determine the haematological parameters and lipid profile of patients with type-2 diabetes and to correlate the results.*
Materials and Methods*: In this prospective cohort study, 30 patients with type-2 diabetes mellitus belonging to age group of 40-80years were recruited. Total white blood cell (TWBC), Neutrophils, Lymphocytes, Monocytes, Eosinophils ,Basophils, Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Platelet (PLT), Red Blood Cell (RBC), Total cholesterol, High density lipoprotein (HDL), Low density lipoprotein (LDL), Triglycerides, Very low density lipoprotein (VLDL) Fasting blood sugar (FBS) and glycated were measured at baseline and 6 month in type-2 diabetes mellitus patients. Twenty five age matched apparently healthy individuals were used as control.*
Results*: At baseline moderate anaemia based on haemoglobin level, neutrophilia, neutropenia, lymphocytosis, lymphopenia, eosinophilia, thrombocytopenia were found. The mean blood levels of total white blood cell and basophils at baseline were significantly higher compared to TWBC and basophils of control subjects (p= 0.010 and 0.039). At baseline, there was strong, positive correlation between basophils and total cholesterol, which was significant (P=0.000). Again at baseline there was a strong positive correlation between neutrophils, monocytes, eosinophils, basophils, RBC, Hb, MCHC and HDL, which was significant (P=0.003, P=0.000, P=0.000, P=0.000, P=0.000, P=0.001, P=0.001 and P=0.000 respectively). In the same way LDL was negatively correlated with neutrophils (P= 0.027), monocytes (P=0.000), eosinophils (P=0.000), RBC (P=0.012), Hb (P=0.000), PCV (P=0.000), MCH (P=0.000) and MCHC (P=0.009) respectively. At six month, total cholesterol was positively correlated with lymphocytes (P=0.021) and MCH (P=0.018) respectively. Also at six month, there was strong, positive correlation between MCV, platelet count and HDL which was significant (P=0.007 and P=0.002 respectively). However, there a significant negative correlation between neutrophils, and low density lipoprotein (P=0.019).*
Conclusion*: From the result obtained, haematological abnormalities were observed at baseline. There was also both strong positive and negative correlation between haematological parameters and lipid profile at baseline and six month.*
Keywords*: baseline; White blood cell; Red blood cell, platelet; Month.*

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I. Introduction

Type 2 diabetes mellitus (DM) is one of the most spread and severe disorder currently, being the fourth mortality reason, globally. It has been reported that number of patients suffering from diabetes mellitus was to be over 200 million people worldwide, a largest part of it being type 2 diabetes mellitus patients [1]. In 2019, diabetes was the major cause of 1.5 million deaths and 48% of all deaths due to diabetes happened before the age of 70 years. There was also a report that from 2000 to 2016, that 5% increase in premature mortality rates occurred before the age of 70 from diabetes. In developed countries the premature mortality rate as a result of diabetes decreased from 2000 to 2010 but then rose in 2010 to 2016. In under developed countries, the untimely death rate due to diabetes increased across both periods [2, 3]. Hyperglycaemia, or high blood sugar, is a

common sign of uncontrolled diabetes and over time leads to serious destruction to many of the body's systems, especially the nerves and blood vessels. Red blood cells (RBCs) are the most glucose-consuming cells. In the presence of diabetes mellitus, the morphology, metabolism, and function of RBCs are unavoidable subject to a series of changes that further affect haemorheology and microcirculation [4]. As an essential component of blood circulation, RBCs is a sensitive index of the body's health status. Some RBCs parameter indicators such as haemoglobin (Hb) concentration, packed cell volume (PCV), and red blood cell count (RBC), can be measured directly from the blood, whereas some parameters, such as mean corpuscular volume (MCV), mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration, can only be calculated from RBCs parameters [5]. These parameters indicate the state of RBCs from various angles and can be basically used to assess the morphology, structure, function, and production of RBCs for the additional diagnosis of some diseases [6, 7]. Study had reported that with an increase in glucose concentration, the periphery of RBCs increased, and the size of RBCs reduced with the increasing irregularity in the RBCs membrane [8]. Finally, when the internal environment of the body changes, the number of normal, biconcave disc RBCs reduced as the number of abnormal RBCs gradually increases, this in addition increases the risk of diabetic complications. Mean corpuscular volume provides information on red cell size and is usually calculated indirectly. Study had reported that MCV is one of the potential risk factor of peripheral artery disease and is associated to disease severity. It can also be used as a prognosis of diabetic macro vascular complications [9]. In conclusion, as convenient, economical and minimally invasive testing methods, MCV in addition to red cell distribution width (RDW) can be also used for the diagnosis of diabetes [9]. Many studies have reported that WBC count, which is routinely measured in laboratory, is related to insulin resistance and increase in WBC can also be a sign for the development of type 2 diabetes mellitus [10, 11]. Diabetes mellitus has been considered as a thrombophilia with increased platelet reactivity [12]. Morphological changes of platelets have been reported and the enhanced platelet activity takes place in diabetic patients [13]. Currently, it is broadly accepted that dyslipidemia is connected with type 2 diabetes mellitus. Those Patients with high triglycerides and low high density lipoprotein levels had 12.75 and 4.89 times increased odds of developing diabetes and prediabetes, respectively [14]. A lipid profile assessment in type 2 diabetes mellitus patients may be useful to decrease the risk of disease progression and also for early intervention. However, few prospective cohort studies have examined the association between haematological parameters and lipid profile in type 2 diabetes mellitus. Thus, this study aimed to determine the haematological parameters and lipid profile of patients with type-2 diabetes and to correlate the results.

II. Material and Methods

This prospective cohort study was carried out on patients of clinic of medical outpatient at Enugu State University of Science and Technology Teaching Hospital (ESUTH) from January to December 2016. Thirty adult subjects (both male and females) of aged between 40-80 years were selected and followed up to 12 month during their treatment period.

Study Location: This was a tertiary care teaching hospital based study done in Haematology laboratory and Chemical pathology laboratory at Enugu State University of Science and Technology Teaching Hospital,

Subjects: Participants were subjects exceeding 40 years of age and diagnosed of type 2 diabetes mellitus at the Chemical Pathology Laboratory of the Enugu State University of Science and Technology Teaching Hospital by glucose oxidase method.

Ethical Approval: Study protocol was approved by Research Ethics committee of Enugu State University of Science and Technology Teaching Hospital (ESUTTH) Enugu

Informed Consent: Each participant in this study signed an informed consent form before blood sample was collected from them.

Laboratory Method: In each subject, 5ml of venous blood samples were obtained after overnight fast into fluoride oxalate tubes (for blood glucose), plain tubes (for lipid profile) and EDTA tubes (for complete blood count and glycated hemoglobin). The complete blood count (CBC) analysis comprising of Hb, PCV, RBC count, TWBC, differential WBC, pLatelet count, MCHC, MCH and MCV were done by coulter method using automated haematology analyzer (Mindray/BC-5150). The lipid profile assay comprising of serum total cholesterol, triglycrides, high density lipoprotein cholesterol were done by the methods based on enzymatic determination using the kits purchased from Randox laboratories Ltd. United Kingdom. Low Density Lipoprotein (LDL) was calculated from friedewald formula. Very low density lipoprotein was calculated by dividing triglyceride value of each patient by five. Glycated hemoglobin was done by the method based on weak binding cation-exchange resin using kit purchased from TECO diagnostics U.S.A.

Statistical analysis: Data was analyzed using SPSS version 21. Comparison of participants' CBC, lipid profile, glycated haemoglobin and fasting blood sugar between baseline and control were done by paired samples t-test. The association of haematological parameters and lipid parameters were done by Pearson correlation. The statistical significance was set at $p<0.05$.

III. Result

At baseline the following red cell, white cell and platelet abnormalities were found. Moderate anaemia based on Hb-3.3%, neutrophilia-6.7%, neutropenia-6.7%, lymphocytosis-6.7%, lymphopenia-6.7%, eosinophilia3.3%, thrombocytopenia-6.7% (Figure 1)

Table no 1 Shows baseline values of haematological parameters, lipid profile, fasting blood sugar and glycated haemoglobin between type 2 diabetes mellitus individuals and apparently healthy control. Individuals with type 2 diabetes mellitus have statistically significant increase in TWBC count, basophils, triglycerides, FBS and glycated haemoglobin (HbA1c) ($p < 0.05$). While the control subjects have significantly higher HDL compared to type 2 diabetes mellitus individuals ($p < 0.05$). Both groups however had similar neutrophils count, lymphocytes count, monocytes count, eosinophils count, RBC count, Hb estimation, PCV, MCV, MCH, MCHC, platelet count, total cholesterol, LDL and VLDL ($p > 0.05$).

Table no 1 show baseline values of haematological parameters, lipid profile, fasting blood sugar and glycated haemoglobin between type 2 diabetes mellitus individuals and apparently healthy control.

Parameters	DM	control	P value
TWBC (X 10^9/l)	5.86±1.63	3.82±0.91	0.010
Neutrophils (%)	51.83±14.79	50.16±10.97	0.607
Lymphocytes (%)	43.30±14.35	44.06±11.84	0.392
Monocytes (%)	2.67±1.24	2.71±1.99	0.952
Eosinophils (%)	3.79±2.50	2.64±1.77	0.087
Basophils (%)	0.54±0.20	0.32±0.17	0.039
RBC (X 10^9/l)	4.48±0.53	4.63±0.51	0.354
Hb (g/dl)	12.65±1.40	12.93±0.64	0.319
PCV (%)	38.10±4.18	38.54±2.14	0.620
MCV (fl)	85.19±4.92	83.89±5.76	0.430
MCH (pg)	28.31±1.76	28.20±2.45	0.860
MCHC (g/dl)	33.24±0.72	33.59±1.36	0.198
Platelet count (X 10^9/l)	186.30±67.32	187.10±43.22	0.955
Total cholesterol (mmol/l)	4.44±1.01	4.97±1.27	0.081
HDL (mmol/l)	1.20±0.75	1.99±0.77	< 0.001
LDL (mmol/l)	2.49±1.27	2.32±1.17	0.614
Triglycerides (mmol/l)	1.74±0.75	1.46±0.23	0.046
VLDL (mmol/l)	0.79±0.34	0.88±0.62	0.526
FBS (mmol/l)	8.02±2.85	5.19±0.88	<0.001
HbA1c (%)	9.24±1.60	6.75±1.75	<0.001

Table no 2 Show the correlation between haematological parameters and lipid parameter of type 2 DM at baseline using Pearson correlation. There was strong, positive correlation between basophils and total cholesterol, which was significant (*P*=0.000). Again, there was strong, negative significant correlation between TWBC, monocytes, eosinophils, Hb, PCV, MCV, MCH, platelet count and total cholesterol (*P*=0.000, *P*=0.001, *P*=0.007, *P*=0.004, *P*=0.003,*P*=0.000, *P*=0.000 and *P*=0.003 respectively). There was a strong positive correlation between neutrophils, monocytes, eosinophils, basophils, RBC, Hb, MCHC and HDL, which was significant (*P*=0.003, *P*=0.000, *P*=0.000, *P*=0.000, *P*=0.000, *P*=0.001, *P*=0.001 and *P*=0.000 respectively). There was also strong, negative significant correlation between TWBC, lymphocytes, MCV, platelet count and HDL (*P*=0.000, *P*=0.000, *P*=0.000 and *P*=0.003 respectively). Low density lipoprotein was positively correlated with TWBC (*P*=0.000) and lymphocytes (*P*=0.000) respectively. In the same way LDL was negatively correlated with neutrophils (*P*= 0.027), monocytes (*P*=0.000), eosinophils (*P*=0.000), RBC (*P*=0.012), Hb (*P*=0.000), PCV (*P*=0.000), MCH (*P*=0.000) and MCHC (*P*=0.009) respectively. Triglycerides showed significant positive correlation with TWBC and basophils (*P*=0.000 and *p*=0.001 respectively). However, there a highly significant negative correlation between monocytes, eosinophil, Hb, MCV, MCH and tiglycerides (*P*=0.000, *P*=0.001, *P*=0.049, *P*=0.000 and *P*=0.000 respectively). Very low density lipoprotein has shown positive correlation with TWBC, while negative correlation with monocytes, eosinophils, MCV and MCH, which were significant.

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Table no 2: Pearson correlation coefficient among the study parameters in type 2 DM at baseline

Parameters	total cholesterol		HDL		LDL		triglycerides		VLDL	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
TWBC	-0.746	0.000**	-0.746	0.000**	0.980	0.000**	0.711	0.000**	0.711	0.000**
Neutrophils	-0.124	0.498	0.503	0.003**	-0.391	0.027*	0.055	0.766	0.079	0.667
Lymphocytes	0.304	0.091	-0.737	0.000**	0.661	0.000**	0.183	0.315	0.167	0.360
Monocytes	-0.538	0.001**	0.600	0.000**	-0.755	0.000**	-0.690	0.000**	-0.711	0.000**
Eosinophils	-0.465	0.007**	0.865	0.000**	-0.848	0.000**	-0.551	0.001**	-0.565	0.001**
Basophils	0.610	0.000**	0.598	0.000**	0.167	0.362	0.457	0.001**	0.430	0.014*
RBC	-0.142	0.440	0.562	0.001**	-0.437	0.012*	0.027	0.885	0.049	0.789
Hb	-0.500	0.004**	0.554	0.001**	-0.718	0.000**	-0.351	0.049*	-0.330	0.065
PCV	-0.512	0.003**	0.288	0.110	-0.582	0.000**	-0.321	0.073	-0.293	0.104
MCV	-9.750	0.000**	-0.611	0.000*	-0.262	0.148	-0.725	0.000**	-0.716	0.000**
MCH	-0.894	0.000**	-0.089	0.629	-0.662	0.000*	-0.961	0.000**	-0.966	0.000**
MCHC	0.044	0.813	0.897	0.000**	-0.455	0.009**	-0.095	0.606	-0.118	0.520
Platelet count	-0.511	0.003**	-0.514	0.003**	-0.135	0.461	-0.135	0.461	-0.321	0.073

Table no 3 Show the correlation between haematological parameters and lipid parameter of type 2 DM at six month using Pearson correlation. Total cholesterol was positively correlated with lymphocytes (P=0.021) and MCH (P=0.018) respectively. In the same way total cholesterol was negatively correlated with neutrophils (P= 0.005). However, total cholesterol did not show significant correlation with monocytes, eosinophils, basophils, RBC, Hb, PCV, MCV, MCHC and platelet count. There was strong, positive correlation between MCV, platelet count and HDL which was significant (P=0.007 and P=0.002 respectively). In the same way, there was negative significant correlation between monocytes, basophils and HDL (P=0.047 and P=0.025 respectively). Low density lipoprotein showed significant positive correlation with eosinophils, basophils and platelet count (P= 0.036, P=0.013 and p=0.019 respectively). However, there a significant negative correlation between neutrophils, and low density lipoprotein (P=0.019). There was positive correlation between RBC, Hb, PCV, platelet count and VLDL (P=0.002, P=0.000, P=0.000 and P=0.041 respectively), which was significant.

Table no 3: Pearson correlation coefficient among the study parameters in type 2 DM at six month

Parameter	total cholesterol		HDL		LDL		triglyceride		VLDL	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
TWBC	-0.401	0.034*	-0.158	0.421	-0.179	0.361	-0.076	0.702	0.166	0.398
Neutrophils	-0.520	0.005**	0.076	0.699	-0.440	0.019*	0.032	0.872	0.062	0.753
Lymphocytes	0.433	0.021*	0.103	0.601	0.251	0.197	-0.030	0.880	0.117	0.553
Monocytes	0.060	0.762	-0.379	0.047*	0.301	0.120	0.072	0.717	-0.331	0.086
Eosinophils	0.261	0.179	-0.286	0.141	0.398	0.036	-0.071	0.721	-0.340	0.076
Basophils	0.229	0.242	-0.422	0.025*	0.465	0.013*	-0.035	0.860	-0.366	0.056
RBC	-0.210	0.282	-0.265	0.173	0.004	0.983	0.154	0.434	0.558	0.002*
Hb	-0.018	0.927	-0.079	0.691	0.041	0.835	-0.004	0.984	0.629	0.000**
PCV	-0.024	0.902	-0.059	0.764	0.023	0.907	0.000	0.999	0.638	0.000**
MCV	0.371	0.052	0.499	0.007**	-0.019	0.923	-0.286	0.141	0.078	0.693
MCH	0.445	0.018*	0.318	0.099	0.155	0.430	-0.299	0.123	0.115	0.560
MCHC	0.093	0.637	0.111	0.573	0.011	0.957	-0.082	0.679	0.029	0.885
Platelet count	-0.056	0.776	0.570	0.002**	0.440	0.019*	-0.094	0.633	0.388	0.041*

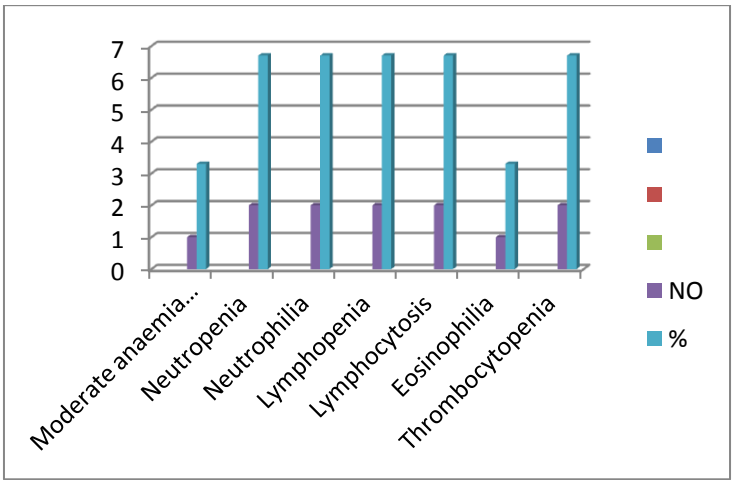

Figure 1: red cell, white cell and platelet abonmalities

IV. Discussion

This study was conducted to follow up selected haematological parameters and lipid profile of individuals with type 2 diabetes mellitus (T2DM) receiving treatment at Enugu State University of Science and Technology Teaching Hospital. This study comprised 30 individuals with T2DM and 25 apparently healthy individuals as controls. Type 2 diabetes mellitus, a chronic disease, is often accompanied by mild to moderate anaemia which is often called anaemia of chronic disease or infection or anaemia of inflammation [15]. In this study moderate anaemia was present in 3.3 % of the patients at baseline which is in line with other studies that found anaemia in T2DM patient [16, 17]. This study defined moderate anaemia as haemoglobin level between 7-10gldl [18]. Studies had shown that diabetes mellitus exerts an adverse effect on almost every aspect of the innate immune system, such as: migratory, phagocytic, oxidative and apoptotic activities, there is also evidence to suggest that diabetes mellitus itself produce a pro-inflammatory background [19, 20]. In this study, various leucocyte abnormalities such as neutropenia,neutrophilia,lymphopenia, lymphocytosis and eosinophilia were observed at baseline. The findings are in agreement with report of previous studies in type 2 diabetes mellitus [21, 22]. Studies, which include in vitro studies, have shown a numerous abnormalities in the mechanisms of action of platelets in diabetic individuals. These abnormalities are responsible for increased sensitivity of platelets to aggregants and decreased sensitivity to antiaggregants and can lead to increased atherosclerosis through increased platelet activity at sites of vessel injury. Alterations in platelets in diabetes comprises increased glycoprotein receptor binding of agonists and adhesive proteins; reduced membrane fluidity; increased activation of the arachidonic acid pathway leading to enhanced thromboxane A2 (TxA_2) formation; changes in phosphatidylinositol (PI) turnover resulting to alterations in diacylglycerol and inositol triphosphate production, calcium mobilization, and protein phosphorylation; abnormal responses to antiaggregants leading in reduced prostacyclin (PGI_2) receptor binding, cyclic nucleotide formation and cyclic nucleotide–dependent protein phosphorylation; and decreased sensitivity to the inhibitory actions of insulin [23, 24]. The above changes lead to abnormal PGI_2 incitement of cyclic AMP (cAMP) and blindness to the inhibitory actions of both PGI_2 and nitric oxide (NO). Platelet dysfunctions combined with reduced endothelial production of these antiaggregatory agents collaborate to increase the risk of cardiovascular disease in patients with type 2 diabetes [25]. In this study, thrombocytopenia was found in two patients (6.7%) at baseline. This finding is in line with study carried by Troussard *et al*., (2014), who reported lower platelet count in elderly patients [26]. The other part of the study examined lipid profile. Also, Pearson-Moment-*r* correlation test was used to investigate the existence of any significant relationship between haematological parameters and lipid parameters among the participants. There was a strong positive correlation between neutrophils, monocytes, eosinophils, basophils, RBC, Hb, MCHC and HDL, which was significant. Also triglycerides showed significant positive correlation with TWBC and basophils. However, LDL was negatively correlated with neutrophils, monocytes, eosinophils, RBC, Hb, PCV, MCH and MCHC. The findings of this study is in line with study done by Samuel *et al*., (2018) in Ghana, a strong, significant positive correlation between RBC and lymphocytes and lipid parameters was observed [27].

V. Conclusion

In this study, various haematological abnormalities such as moderate anaemia, neutropenia, neutrophilia, lymphopenia, lymphocytosis, eosinophilia and thrombocytopenia were observed at baseline. There was also both strong positive and negative correlation between haematological parameters and lipid profile at baseline and six month.

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