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"A Study of Hematological Parameters and Their Correlation with Blood Pressure and Renal Function Test Among Type-2 Diabetes Mellitus"

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ABSTRACT:

Background: Diabetes Mellitus is part of a metabolic syndrome comprises obesity, dyslipidemia, hypertension, and the changes in haematological parameters. Uncontrolled type 2 Diabetes Miletus (T2DM) is associated with multiple disorders including cellular, metabolic, and the blood disturbances leading to the vascular complications.
Aim: To study of the hematological parameters and their correlation with blood pressure and renal function test among type-2 diabetes mellitus.
Methodology: The present comparative cross-sectional study performed in Department of Physiology and Medicine of Integral Institute of Medical Sciences and Research, Lucknow. 70 uncontrolled DM (HbA1c >7.0%) either sex adult age group 30 to 70 years patients and 70 control DM (HbA1c <7.0%) of the same age and sex patients were enrolled in this study. eGFR was calculated by CKD-EPI creatinine equation (2021).
Result: There were significantly elevated SBP, DBP, S. Creatinine, eGFR and RDW level in uncontrolled DM group ($p<0.5$). While Hb, RBC, TLC, MCV, MCH, MCHC and Platelet count level significantly decrease in uncontrol DM group ($p<0.05$). There was a strong correlation between diabetes profiles, blood pressure and kidney function in DM patients. In uncontrolled diabetes group MCV, MCH, MCH and Platelet counts were negatively significant correlation with SBP, DBP, S.creatinine and eGFR.
Conclusion: RBC indices (MCV, MCH, MCH and Platelet counts) in uncontrol DM group was negatively significant correlation with FBS, PPBS, SBP, DBP and S, creatinine; but positive significant correlation with eGFR.

Keywords: Diabetes Mellitus, Glycosylated Hemoglobin, Blood Pressure, Renal Function

1. Introduction:

Chronic metabolic disease known as diabetes mellitus (DM) is typified by elevated blood sugar levels, which can cause serious harm and malfunction to the heart, kidneys, blood vessels, eyes, and nerves. There are two primary kinds of diabetes mellitus: Type-1 DM, which is autoimmune in nature and arises from the death of β-cells in the pancreas, causing abnormalities in insulin synthesis, and Type-2 DM, which is adult-onset and comes from either insufficient or resistant insulin production [1]. Both lead to decrease or the prevention of production of insulin, which results in group of the metabolic imbalances go together with multiple disorders in metabolism of lipid, protein and carbohydrate [2]. DM is convoyed by hyperglycaemia, hyperlipidaemia, and glycosuria.

Uncontrolled DM is related with multiple disorders with cellular, metabolic, and the blood disturbances leading to the vascular complications [3]. T2DM is part of a metabolic syndrome comprises obesity, dyslipidaemia, hypertension, and the changes in haematological parameters. Changes in the activity and metabolism of red blood cells (RBCs), white blood cells (WBCs), platelets (PLTs), and coagulation systems are among the haematological abnormalities associated with type 2 diabetes (T2DM) [4]. In comparison to those without diabetes, these alterations may show up as immunological and coagulation issues as well as anaemia, which is defined by a drop in the RBC count, haemoglobin (Hgb), and hematocrit (Hct) level. Estimates of the prevalence of anaemia, a common and sometimes undiagnosed haematological alteration in people with type 2 diabetes, vary greatly [5].

DM is shown to be directly associated with several haematological changes affecting the RBCs [6]. Hyperglycemia promotes the steady development of glycosylated Hb, which is associated with osmotic disruption, cytoplasmic viscosity, and structural and functional changes in the Hgb molecule. Any of the RBC indices, such as the red cell distribution width (RDW), mean cell haemoglobin (MCH), MCHC, Hct, and RBC count, might be adversely affected by all of these alterations [7].

Numerous variables, such as increased reactive oxygen species (ROS) generation and the development of advanced glycation end products (AGEs) as a result of persistent hyperglycemia, can lead to haematological abnormalities in diabetes. Oxidative stress, which is linked to tissue damage and haematological abnormalities such RBC malfunction, PLT hyperactivity, and endothelial dysfunction, is brought on by an increase in ROS production [8]. These haematological changes might cause complications like anaemia, and state of hyper-coagulability, and contribute to the cardiovascular disease in diabetic patients [9]. A further mechanism that quickens the vascular problems in T2DM patients is insulin resistance, which is linked to endothelial dysfunction, elevated inflammatory marker levels, and PLT hyperactivity [8].

Haematological measures like WBC, RDW, platelet distribution width, mean platelet volume (MPV), and platelet count have sparked interest once again since they can be used as indicators of inflammation and endothelial dysfunction in type-2 diabetes [10]. A higher WBC count is a traditional indicator of inflammation, and epidemiological research indicates a link between diabetes risk and WBC count. The stability of normal homeostasis depends heavily on platelets, and MPV serves as a measure for platelet function [11]. Diabetes is made more difficult by accelerated atherosclerosis. In patients with T2DM, platelet activation is important for inflammation and atherothrombosis process plays a part in development of CVD. Increased MPV been seen in diabetic individuals with nephropathy, coronary heart disease, and retinopathy. MPV is a measure of changes in either platelet stimulation or rate of platelet generation [12].

In general, T2DM patients have been shown to have haematological abnormalities. Periodic follow-up of haematological markers is not recommended by current diabetes care

recommendations. Even though several investigations on the haematological parameters of diabetes individuals produced a variety of contradicting data. WBC, RBC, and PLT are higher significantly in diabetics than controls, according to one study, while other studies found insignificant difference in these parameters between diabetics and healthy controls [13]. According to other reports, the diabetic group had considerably greater PLT and indices than controls, whereas all RBC indices except RDW were significantly lower. Furthermore, there is a dearth of data in India, especially when it comes to research on the relationship between decreased renal function and haematological markers in T2DM patients. Hematologic analyzer parameters, such as haemoglobin, RBC, TLC, MCH, MCHC, MCV, RDW, and platelet parameters, can provide light on changes that take place in haematological indices. Thus, the purpose of this study was to evaluate blood pressure, haematological parameters, and their relationship to a renal function test in individuals with type 2 diabetes.

Aim: To study of the hematological parameters and their correlation with blood pressure and renal function test among type-2 diabetes mellitus.

2. Material & Methods:

The present comparative cross-sectional study performed in Department of Physiology and Medicine of Integral Institute of Medical Sciences and Research, Lucknow. 70 uncontrolled diabetes (HbA1c >7.0%) either sex adult age group 30 to 70 years patients and 70 controlled diabetes (HbA1c <7.0%) of the same age and sex patients were enrolled in this study. The research excluded patients with a history of chronic liver illness, including hepatitis B and hepatitis C viruses, heart failure, cancer, bleeding problems, infectious infections, and pregnancy.

Hypertension was diagnosed if there was a prior diagnosis by a physician, or measured blood pressure values of ≥140 mmHg systolic or ≥90 mmHg diastolic on ≥2 occasions [14].

Samples of venous blood were drawn from each participant. Following an overnight fast, laboratory personnel took a six-milliliter venous blood sample from each T2DM case (2 ml in serum separator tube, and 4 ml in an EDTA tube). Conversely, at the moment of donation, four millilitres of venous blood were drawn from the blood donor control group and placed into an EDTA test tube. Serum samples were obtained by centrifugation and the biochemical parameters were measured. Quantitative determination of HbA1c estimation was done using D-10 Fully automated analyzer. Biochemical analysis: Serum Creatinine, Serum albumin, serum urea, fasting and post prandial blood sugar was assessed by (SIEMENS, DIMENSIONS RxL MAX automatic analyzer) in central pathology. Hematological parameters (hemoglobin, RBC, white blood cell (WBC), platelet count, red cell distribution width (RDW), will be assessed from CBC done by NIHON KOHDEN fully automatic analyzer in central pathology. eGFR will be calculated by CKD-EPI creatinine equation (2021). eGFRcr = 142 * min (Scr /k,1) a * max (Scr /k,1)-1.200 * 0.9938age * 1.012 (if female) Scr = standard serum creatinine in mg/dl

Statistical analysis:

Microsoft Excel sheet used to create the database and generating graphs, while data was assessed using Statistical Package for the Social Sciences (SPSS) version 23.0 for Windows. Categorical/Ordinal data was expressed as percentage and compare by using Chi Square test. Independent Sample t test was used to test difference between quantitative data among groups. Bivariate analysis (Pearson correlation) was used to find the association of haematological profile (haemoglobin, RBC, TLC, MCV, MCH, MCHC, RDW and Platelet parameters), with blood pressure (SBP, DBP), renal function test (S. creatinine level, eGFR)

and diabetes profile (FBS, PPBS and HbA1c). A p-value less than 0.05 (P<0.05) was considered statistically significant.

3. Result:

In **table no 1** we noted that the age and sex of the studied were insignificant distribution in both groups. In our study uncontrolled diabetes cases we find the significantly elevated SBP, DBP, S. Creatinine, eGFR and RDW level in uncontrolled DM group (p<0.5). While Hb, RBC, TLC, MCV, MCH, MCHC and Platelet count level significantly decrease (p<0.05 in uncontrolled DM group.
In **table no 2** we noted that there was a strong correlation between blood pressure and renal function in diabetic patients.
In **table no 3** we noted that MCV, MCH, MCH and Platelet counts in uncontrolled DM group was negatively significant correlation with systolic and diastolic blood pressure. RBC and RDW in uncontrolled DM group were positively significantly associated with diastolic blood pressure. While haemoglobin and TLC was insignificantly association with diastolic blood pressure in uncontrolled DM group. But in control diabetes group only TLC was negatively significant correlation with systolic and diastolic blood pressure. Rest others haematological parameters in uncontrolled diabetes group were insignificant association with diastolic blood pressure.
In **table no 4** we noted that MCV, MCH, MCH and Platelet counts in uncontrol DM group was negatively significant correlation with s. creatinine and eGFR. RBS and RDW (cv) in uncontrol DM group was positive significantly associated with s. creatinine and eGFR. While TLC was negative significant association with serum creatinine in uncontrol DM group. Rest others haematological parameters in uncontrol DM group and several haematological parameters in control DM group were insignificant association with S. Creatinine and eGFR.

Table 1: Comparison of hematological parameters between uncontrolled and controlled diabetes

		Group		**P value**
		Uncontrolled Diabetes (n=70)	**Controlled Diabetes (n=70)**	
Sex	Male	39 (55.7%)	36 (51.4%)	0.735#
	Female	31 (44.3%)	34 (84.6%)	
Age		52.46±11.802	49.23±9.27	0.074*
SBP		136.27±14.27	121.23±12.27	**<0.001***
DBP		87.94±9.78	80.14±7.82	**<0.001***
FBS		160.66±31.06	136.94±11.71	**<0.001***
PPBS		236.17±48.54	208.46±28.85	**<0.001***
HbA1c		9.98±1.92	6.34±0.52	**<0.001***
S. Creatinine		2.52±0.81	1.58±0.41	**<0.001***
eGFR		31.93±9.2610.58	56.09±18.57	**<0.001***
Hb		11.60±0.92	12.32±0.94	**<0.001***
RBC		4.33±0.67	4.55±0.44	**0.022***
TLC		8122.71±1372.12	9038.57±1983.58	**0.002***
MCV		83.67±5.11	87.17±5.08	**<0.001***
MCH		26.26±2.66	28.40±2.61	**<0.001***
MCHC		30.93±1.93	32.92±1.76	**<0.001***
RDW cv		15.25±2.79	13.25±1.07	**<0.001***
Platelet		2.23±0.69	2.44±0.56	**0.003***

#Chi Square test; *Independent Sample t test;
[SBP=Systolic Blood Pressure; BBP=Diastolic Blood Pressure; FBS=Fasting Blood Sugar; PPBS=Post Prandial Blood Sugar; HbA1c=Glycosylated Hemoglobin; eGFR=Estimated Glomerular filtration Rate; HB=Hemoglobin; RBC=Red Blood Corpuscle; TLC=Total Leukocyte Count; MCV= Mean Corpuscular Volume; MCH= Mean Corpuscular Hemoglobin; MCHC= Mean Corpuscular Hemoglobin Concentration; RDW-cv=Red Cell Distribution Width - Coefficient of Variation]

Table 2: Correlation matrix of diabetes level, blood pressure and renal function

		SBP	**DBP**	**FBS**	**PPBS**	**HbA1c**	**S. Creatinine**	**eGFR**
SBP	r value	1	0.602**	0.358**	0.416**	0.380**	0.619**	-0.471**
	p value		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
DBP	r value	0.602**	1	0.420**	0.419**	0.332**	.486**	-0.367**
	p value	**<0.001**		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
FBS	r value	0.358**	0.420**	1	0.564**	0.322**	0.525**	-0.428**
	p value	**<0.001**	**<0.001**		**<0.001**	**<0.001**	**<0.001**	**<0.001**
PPBS	r value	0.416**	0.419**	0.564**	1	0.242**	0.467**	-0.362**
	p value	**<0.001**	**<0.001**	**<0.001**		**0.004**	**0.000**	**0.000**
HbA1c	r value	0.380**	0.332**	0.322**	0.242**	1	0.377**	-0.446**
	p value	**<0.001**	**<0.001**	**<0.001**	**0.004**		**<0.001**	**<0.001**
S. Creatinine	r value	0.619**	0.486**	0.525**	0.467**	0.377**	1	-0.857**
	p value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**		**<0.001**
eGFR	r value	-0.471**	-0.367**	-0.428**	-0.362**	-0.446**	-0.857**	1
	p value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	
**. Correlation is significant at the 0.01 level (2-tailed).								

*Bivariate analysis (Pearson correlation)
r value= Pearson's correlation coefficients; p value=Significance level;

Table 3: Correlation of hematological parameters with blood pressure

		Control Diabetes		**Uncontrol Diabetes**	
		SBP	**DBP**	**SBP**	**DBP**
Hb	r value	0.171	0.061	-0.178	-0.235
	p value	0.157	0.616	0.140	0.050
RBC	r value	-0.028	-.0219	0.493**	0.149
	p value	0.815	0.068	**<0.001**	0.218
TLC	r value	-0.258*	-0.126	0.079	-0.035
	p value	**0.031**	0.297	0.516	0.775
MCV	r value	0.116	0.116	-0.327**	-0.233
	p value	0.338	0.341	**0.006**	0.052
MCH	r value	-0.077	-0.173	-0.291*	-0.237*
	p value	0.524	0.151	**0.015**	**0.048**
MCHC	r value	-0.164	-0.049	-0.444**	-0.376**
	p value	0.175	0.686	**<0.001**	**<0.001**
RDW (cv)	r value	0.171	0.216	0.511**	0.315**
	p value	0.156	0.073	**<0.001**	**0.008**

Platelet	r value	-0.126	-0.064	-0.749**	-0.610**
	p value	0.299	0.597	**<0.001**	**<0.001**
**. Correlation is significant at the 0.01 level (2-tailed).					
*. Correlation is significant at the 0.05 level (2-tailed).					

*Bivariate analysis (Pearson correlation)
r value= Pearson's correlation coefficients; p value=Significance level;

Table 4: Correlation of hematological parameters with renal function

		Control Diabetes		**Uncontrol Diabetes**	
		S. Creatinine	**eGFR**	**S. Creatinine**	**eGFR**
Hb	r value	0.062	-0.051	-0.079	0.080
	p value	0.612	0.677	0.518	0.509
RBC	r value	-0.166	0.099	0.497**	-0.475**
	p value	0.169	0.413	**<0.001**	**<0.001**
TLC	r value	0.046	-0.046	-0.240*	0.160
	p value	0.705	0.703	**0.045**	0.185
MCV	r value	0.074	-0.095	-0.323**	0.319**
	p value	0.545	0.432	**0.006**	**0.007**
MCH	r value	0.078	-0.058	-0.420**	0.407**
	p value	0.522	0.632	**<0.001**	**<0.001**
MCHC	r value	-0.065	0.086	-0.474**	0.509**
	p value	0.593	0.477	**<0.001**	**<0.001**
RDW(cv)	r value	0.000	0.016	0.619**	-0.533**
	p value	0.997	0.894	**<0.001**	**<0.001**
Platelet	r value	0.057	-0.060	-0.673**	0.727**
	p value	0.640	0.624	**<0.001**	**<0.001**
**. Correlation is significant at the 0.01 level (2-tailed).					
*. Correlation is significant at the 0.05 level (2-tailed).					

*Bivariate analysis (Pearson correlation)
r value= Pearson's correlation coefficients; p value=Significance level;

4. Discussion:

Diabetes mellitus is chronic disease marked by high blood sugar levels because the body is unable to effectively use or create adequate insulin. Significant abnormalities were seen in a number of haematological indicators in DM patients [2,15]. This study involved comparison of FBS and PPBS levels between uncontrol DM group and control DM group. We noted significant alteration in FBS and PPBS levels between uncontrol DM group and control DM group ($P<0.0001$). The study also included a comparison of blood pressure (BP) between the two groups, and the findings of this comparison revealed significant differences in both SBP and DBP ($p<0.0001$). This result was in concordance with **Abbas AB et al** [16] and **Biadgo Bet al [6]**. This was because of direct and the toxic effects of the chronic hyperglycaemia on the endothelial cells of vascular which cause vascular repairs and the increased vasoconstriction ultimately affecting BP [17].

In this study, Independent Sample t-test displayed that mean and SD of uncontrol diabetes cases we find the significantly elevated SBP, DBP, S. Creatinine, eGFR and RDW level ($p<0.5$). While hemoglobin, RBC, TLC, MCV, MCH, MCHC and Platelet count level significantly decrease ($p<0.05$). Numerous research undertaken across various nations have

demonstrated variances in hematologic indices; a few these studies have demonstrated relevance, while others have not. Between the groups DM and HC in India, Nigeria, and Sudanthere were statistically significant differences in monocyte, RBCs, basophil, MCH, and MCV [2,18,19,20]. The findings of monocyte and the basophil in Nigeria were low significantly in DM than HC group [20]; while in other studies in Ethiopia, Turkey, and India where monocyte was high statistically in DM than HC group [21,22]. The results of MCV and MCH in Brazil, Saudi Arabia, Ethiopia, India, and the Sudan were low statistically in DM than HC group [7,23,24,25]. In dissimilarity, study in Saudi Arabia exhibited that the MCHC was high statistically in DM than HC group [7], In this study further we noted that the MCHC and Platelet counts in uncontrol diabetes group was negatively significant correlation with fasting and post prandial blood sugar. Haemoglobin in uncontrol diabetes group was positive significantly associated with FBS. Rest others haematological parameters in uncontrol diabetes group and several haematological parameters in control diabetes group were insignificant association with diastolic blood pressure. In table we also noted that there was no any significant correlation of between haematological parameters and HbA1c in both control and uncontrol diabetes groups.

In T2DM cases, hypertension often heads albuminuria, and eGFR declines [26]. Hypertension co-exists with the other CV risk-factors like dyslipidaemia, and the obesity [27]. These risk variables have the potential to worsen systemic hypertension and may both contribute to and result from the decline in renal function. Proteinuria and hyperfiltration are brought on by systemic hypertension, which raises intraglomerular pressure [28]. Furthermore, over-production of the vasoactive factors disrupting the renal vascular auto-regulation worsens the glomerular hyperfiltration, where RAAS activation plays crucial role [29.30]. The development of the path gnomonic characteristics of DKD, including glomerular hypertrophy and sclerosis, tubulointerstitial inflammation, and fibrosis, is facilitated by the local generation of angiotensin II, which also causes intraglomerular hypertension, proteinuria, and inflammatory pathways [31]. Current study noted that there was a strong significant correlation between diabetes profile (FBS, PPBS and HbA1c), blood pressure (SBP and DBP) and renal function (Serum Creatinine and eGFR) in diabetic patients.

By using the Bivariate analysis (Pearson correlation, we noted that MCV, MCH, MCHC and Platelet counts in uncontrol diabetes group was negatively significant correlation with systolic and diastolic blood pressure. RBS and RDW (cv) in uncontrol diabetes group was positive significantly associated with DBP. While haemoglobin and TLC was insignificant association with diastolic blood pressure in uncontrol diabetes group. But in control diabetes group only TLC was negatively significant correlation with SBP and DBP. Rest others haematological parameters in uncontrol diabetes group were insignificant association with DBP. **Adane T et al [32]** reported that in DM patients, they discovered a positive correlation between DBP and MCV and MCH. Development of D Mrelated hypertension and dyslipidaemia in those individuals may be the cause of the link seen between blood pressure, and RBC parameters [17].

Our study noted that the MCV, MCH, MCHC and Platelet counts in uncontrol diabetes group was negatively significant correlation with s. creatinine and eGFR. RBS and RDW (cv) in uncontrol diabetes group was positive significantly associated with s. creatinine and eGFR. While TLC was negative significant association with serum creatinine in uncontrol diabetes group. Rest others haematological parameters in uncontrol diabetes group and several haematological parameters in control diabetes group were insignificant association with S. Creatinine and eGFR**. Vintha RK et al** [33] reported that the majority of patients with Grade 2 and Grade 3 CKD had mildto-moderate grades of anaemia which worsened as the stage of CKD progressed and came out to be highly significant. **Adane T et al [31]** said that in individuals with diabetes, a strong negative connection was seen between the serum

creatinine levels and the Hct, RBC, Hgb, and MCV. Our results were consistent with those of research conducted in Nigeria and Cameroon, which found a substantial negative connection between serum creatinine and haemoglobin [34]. The reason for the negative connection between Hgb and Cr in diabetes mellitus is that people with renal disease tend to have higher blood creatinine and BUN levels when their kidneys are not working correctly. Damage to the peritubular fibroblasts in people with diabetes mellitus might result in low Hgb and anaemia due to E deficiency [35].

Limitations of the study:
First off, because the study was cross-sectional in nature, it was challenging to determine a cause-and-effect link between DM and RBC parameters. Second, because of resource constraints, the study did not examine measures such serum iron, ferritin, and vitamin B12. Therefore, it was not intended to evaluate the kind of anaemia that individuals with diabetes mellitus present with.

5. Conclusion:

The mean RBC parameters Hb, RBC, TLC, Neutrophils, Lymphocyte, MCV, MCH, MCHC and Platelet count level for uncontrol DM cases were lower significantly than control DM patients. RBC indices (MCV, MCH, MCH and Platelet counts) in uncontrol diabetes group was negatively significant correlation with systolic and diastolic blood pressure. Besides significant negative association between serum creatinine and RBC indices (MCV, MCH, MCH and Platelet counts) while it was found significant positive correlation with eGFR in uncontrolled DM patients. It is, thus, suggested that RBC parameters abnormalities must be evaluated, and periodically treated in uncontrol DM cases for the better prognosis and the quality of life.

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