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EVALUATION OF CORRELATION BETWEEN GLYCATED HEMOGLOBIN AND HEMATOLOGICAL INDICES IN TYPE 2 DIABETES MELLITUS PATIENTS

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Keywords:

HbA1c, Mean Corpuscular Haemoglobin Concentration, Red cell Distribution Width, Mean platelet volume, White blood cells, Hyperlipidemia, poorly controlled diabetes, Inflammation, cardiovascular risk

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ABSTRACT: Diabetes Mellitus increases the risk of both microvascular and macrovascular complications, out of which CVD affects approximately 32.2% of people with Type 2 Diabetes Mellitus globally. It is predicted that for every 1% increase in HbA1c is associated with a 30% increase in all-cause mortality and a 40% increase in CVD mortality in T2DM patients. This study aims to evaluate the correlation of HbA1c% with various hematological indices and to elucidate the possible changes in some hematological and lipid parameters in people with poor glycoregulation. A total of 76 patients of both gender with type 2 DM were included in the study after fulfilling the exclusion and inclusion criteria. Data from complete blood picture reports of the subjects were collected and documented. The collected data includes the values of HbA1c, some elements of complete blood picture, and lipid profile. The subjects were divided into two categories with $HbA1c\% \leq 7$ and $HbA1c\% > 7$. All blood parameters under study except lymphocytes showed a significant mean increase in subjects with $HbA1c\% > 7$. HbA1c showed a statistically significant positive correlation with MCHC ($r=0.310$), RDW ($r=0.584$), WBC ($r=0.453$), MPV ($r=0.709$), TC ($r=0.471$), TG ($r=0.426$) and VLDL ($r=0.437$) at p -value < 0.05 . Neutrophils and MCH showed no significant positive correlation, and lymphocytes count was negatively correlated with HbA1c. Our study concluded that HbA1c% was positively correlated with MCHC, RDW, MPV, WBC, TC, TG, and VLDL. Hyperlipidemia was also found to be persistent among patients with poor glycemic status, which is associated with a higher risk of cardiovascular-related mortality and morbidity.

INTRODUCTION: Diabetes Mellitus is a non-communicable chronic metabolic disorder in which blood sugar (glucose) levels are abnormally high (hyperglycemia) as the result of a defect in insulin secretion, insulin resistance, or both ¹.

The most common type of Diabetes Mellitus is Type 2 Diabetes Mellitus which accounts for around 90% of cases worldwide ². Globally, the prevalence of Type 2 Diabetes Mellitus is increasing across all the regions which is driven by increasing urbanization, population aging, sedentary lifestyles, and greater consumption of unhealthy foods linked with obesity.

According to IDF-9th edition, it is estimated that 463 million adults of age 20-79 years are currently living with Diabetes Mellitus, which is about 9.3%

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of the world population in this age group. The total number is predicted to rise to 578.4 million (10.2%) by 2030 and to 7000.2 million (10.9%) by 2045. The total number of deaths resulting from diabetes and its complications in 2019 is estimated to be 4.2 million. Diabetes Mellitus increases the risk of both microvascular and macrovascular complications, out of which CVD affects approximately 32.2% of people with Type 2 Diabetes Mellitus globally³.

HbA1c is a clinical standard measure of blood sugar management. HbA1c is the best available biochemical parameter that provides a long-term trend of how high blood glucose levels have been for the period of the last 6-8 weeks. It is closely associated with the risk of developing complications and hence it is used as an evidence-based marker to assess the chances of developing diabetic complications⁴. It is predicted that for every 1% increase in HbA1c is associated with a 30% increase in all-cause mortality and a 40% increase in CVD mortality in T2DM patients⁵.

Many studies revealed that hematological indices and lipid parameters role in assessing the risk of vascular complications. Red cell distribution width (RDW) is a recently described as a novel inflammatory biomarker to predict cardiovascular mortality, particularly in people with poor glycemic control and also in renal diseases⁶. RDW is a measure of anisocytosis and there are no possible well-established pathological mechanisms to explain the relationship between cardiovascular outcomes and RDW, but both oxidative stress and inflammation are believed to play a prominent role⁷.

WBC, a marker of inflammation, predicts a worsening of insulin action, insulin secretory function⁸, and the development of micro and macrovascular complications⁹. Activation of pro-inflammatory signalling pathways that promote the differentiation and maturation of WBCs are involved in the pathogenesis of insulin resistance, *via* various cytokines and signalling pathways¹⁰. Elevated WBC is also associated with atherosclerotic changes which lead to cardiovascular complications¹¹.

MPV is used to estimate the platelet function and aggregation potential, which plays a key role in thrombogenesis and atherosclerosis¹² and might be a useful prognostic marker of cardiovascular

complications in diabetic patients^{13, 14}. It was found that diabetic patients have large platelets that produce more β -thromboglobulin, serotonin, and produce more thromboxane than normal people¹³ and contain less antioxidants¹⁵. Hyperglycemia and hyperinsulinemia might regulate the production of interleukin-6 and thrombopoietin that leads to an increase in platelet size¹⁶.

Hyperlipidemia is more prevalent among Type 2 diabetes patients and a modifiable risk factor for cardiac problems. Insulin resistance or deficiency affects the key enzymes and pathways in lipid metabolism leading to lipid abnormalities among type 2 diabetes patients¹⁷. Inflammation caused by glycated hemoglobin is responsible for the formation of atherosclerotic plaque (atheroma)¹⁸. In insulin-resistant conditions, hepatic triglycerides synthesis increases in diabetic patients due to the antilipolytic effect of insulin and also increase in free fatty acids¹⁹.

We aimed to evaluate the correlation between HbA1c and various hematological indices. To elucidate the possible changes in some hematological and lipid parameters in people with poor glycemic control. Hematological indices that are claimed to have a role in inflammation were considered for the study.

MATERIALS AND METHODS:

Study Design and Location: This is a prospective cross-sectional observational study conducted at West Godavari District Government Hospital, Eluru from July 2019 to December 2019. A total of 76 patients (females 41, males 35) with type 2 DM visiting the outpatient department (Non-Communicable Disease clinic) of District Government Hospital, Eluru were included in the study after obtaining informed consent. Our study is supported by Dr. B. Anand Kumar (General Physician, Internal Medicine), Dr. V. Yogendra Babu (Civil Assistant Surgeon, General Medicine) under the guidance of Kumaraswamy Barla (Assistant Professor, M. Pharm, Pharmacology). Participants were enrolled in the study after fulfilling the diagnostic criteria by the American Diabetes Association.

Criteria for Selection of Patients:

Inclusion Criteria: (1) Both males and females with type 2 DM of age above 30 years, who agreed

to participate in the study, (2) Patients with BMI \leq 30, (3) Patients without any other systemic medical condition prior to DM onset, (4) Subjects who were on regular medication.

Exclusion Criteria: (1) Subjects on Anti-platelet drugs such as aspirin and clopidogrel and anti-hyperlipidemic drugs such as statins and fibrates, (2) subjects with cardiovascular problems, (3) thyroid problems, (4) newly diagnosed T2DM patients (5) type 1 diabetes, (6) renal and hepatic impairment and (7) subjects with haematological diseases, (8) impaired movement and communication disability, (9) acute infections, malignancy were excluded from the study.

Questionnaire: The patient-specific data was collected by using an interview-guided self-administered questionnaire. We approached the patients by oral questioning regarding detailed Socio-demographic data, Behavioural factors, Medical-related factors, and management of clinical factors.

We collected the complete blood picture laboratory test data from a total of 76 subjects with type 2 DM. The laboratory test data included the parameters HbA1c, complete blood picture, total cholesterol, VLDL, and triglycerides of the subjects. The data collected was documented.

After baseline evaluation, diabetic patients were divided into two groups according to their HbA1c levels. Group A consisted of 29 patients with HbA1c levels \leq 7%, and group B consisted of 47 patients with HbA1c levels $>$ 7.

Our article has been approved by IRB (Institutional Review Board) IRB NO: SIRCRRCOPS/IRB/2019-2020/02.

Statistical Analysis: Statistical analysis and data documented were analyzed by using Microsoft excel 2013. Pearson's correlation test was performed to examine various correlations. Independent sample Student's t-test (two-tailed) was used to compare the means of different parameters. All p values less than 0.05 were considered to be statistically significant. All values are expressed as mean \pm standard deviation (SD) or standard error of the mean.

RESULTS: A total of 76 subjects were enrolled in the study after evaluating their laboratory data. Out of 76 subjects, 35 were males and 41 were females. Based on the HbA1c%, subjects were grouped into two categories, Group A (n=29; males=13, females =16) and Group B (n=47, males = 22, females = 25). The mean age difference between subjects in Group A and Group B (55.13 ± 6.37 and 55.97 ± 9.8) was not significant. There was a significant mean difference in duration of T2DM between both Group A (6.04 ± 4.00) and Group B (8.2 ± 14.79) at p-value 0.03.

In Group A and Group B, subjects taking only metformin were (24.1% and 0%), metformin and insulin were (6.1% and 17.02%), metformin and glimepiride were (68.9% and 46.8%) and a combination of metformin, glimepiride, insulin were (0% and 25.3%).

TABLE 1: BASELINE CHARACTERISTICS OF THE POPULATION UNDER STUDY

	Diabetic population (n=76)				p-value
	HbA1c \leq 7		HbA1c $>$ 7		
	n	n%	n	n%	
Total subjects	29	38.1%	47	61.8%	--
Gender	male = 13 Female = 16	44.8% 55.1%	Male = 22 Female 25	46.8% 53.1%	--
Age (mean \pm S.D)	55.13 ± 6.37	----	55.97 ± 9.8	----	0.59
Duration of DM	6.04 ± 4.00	----	8.2 ± 14.79	----	0.03
Metformin	7	24.1	0	0%	--
Metformin + Insulin	2	6.1	8	17.02%	--
Metformin + Glimepiride	20	68.9	22	46.8%	--
Metformin + Glimepiride + insulin	0	0%	12	25.3%	--

Data is expressed as mean \pm SD. Mean Corpuscular Volume, Mean Corpuscular Hemoglobin Concentration, Red cell Distribution Width, White Blood cells, neutrophils, Mean Platelet Volume, Total cholesterol, Triglycerides, Very low-density lipoprotein showed a significantly higher mean in subjects with HbA1c % above 7, at p-value $<$ 0.05.

TABLE 2: EVALUATION OF THE MEAN DIFFERENCE OF PARAMETERS BETWEEN SUBJECTS WITH GOOD GLYCEMIC CONTROL AND POOR GLYCEMIC CONTROL

Parameters	HbA1c ≤7 Mean ± SD	HbA1c >7 Mean± S.D	p-value
MCH (pg/red cell)	28.63 ±2.21	31.12±3.20	0.000
MCHC (g/dl)	32.06±1.49	33.42±1.87	0.000
RDW (%)	13.1±1.82	14.2±1.76	0.012
WBC (cells/mm ³)	7467.17 ±1276.51	9031.10 ±1942	0.000
Neutrophils %	53.61±5.01	58.11±5.98	0.000
Lymphocytes %	35.21±6.49	32.18±5.66	0.034
MPV (fL)	8.40 ± 0.63	10.02±1.72	0.000
Total cholesterol (mg/dl)	165.96±28.24	188.46±30.20	0.001
Triglycerides (mg/dl)	141.58±37.96	183.59±42.80	0.000
VLDL (mg/dl)	27.94±7.46	36.49±8.51	0.000

P-value is considered significant at < 0.05

Abbreviations: Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Red cell Distribution Width (RDW), White Blood cells (WBC), Mean Platelet Volume (MPV), Total cholesterol (TC), Triglycerides (TG), Very Low-Density Lipoprotein (VLDL)

TABLE 3: CORRELATION ANALYSIS OF HbA1c% WITH MCH, MCHC, RDW, WBC, NEUTROPHILS, LYMPHOCYTES, MPV, TC, TG, AND VLDL OF THE WHOLE SAMPLE

	HbA1c	
	Correlation (r)	p-value
MCH	0.185	0.107
MCHC	0.310**	0.000
RDW-CV	0.584**	0.000
WBC	0.453**	0.000
Neutrophils	0.211	0.067
Lymphocytes	-0.13	0.262
MPV	0.709**	0.000
TC	0.471**	0.000
TG	0.426**	0.000
VLDL	0.437**	0.000

**Correlation significant

The correlation analysis between HbA1c and parameters of blood elements under study is illustrated in **Table 3**. MCHC, RDW, MPV, WBC, and lipid parameters showed a significant positive relation with increasing HbA1c%. MCH and neutrophils showed no significant positive relation, and Lymphocytes showed a negative relationship with HbA1c%.

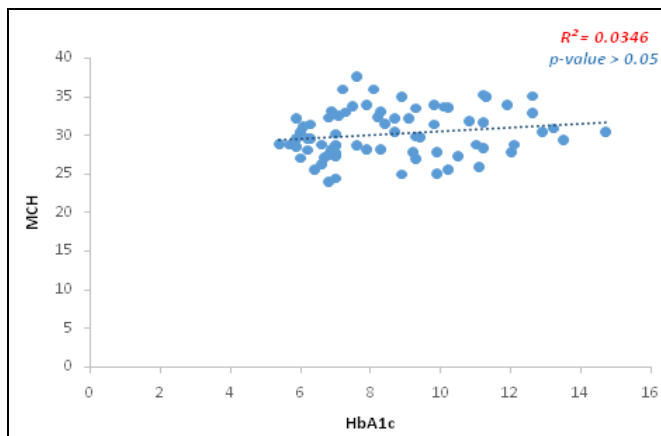


FIG. 1: SCATTER PLOT GRAPH OF MCH AND HbA1c LEVELS IN THE TOTAL SAMPLE

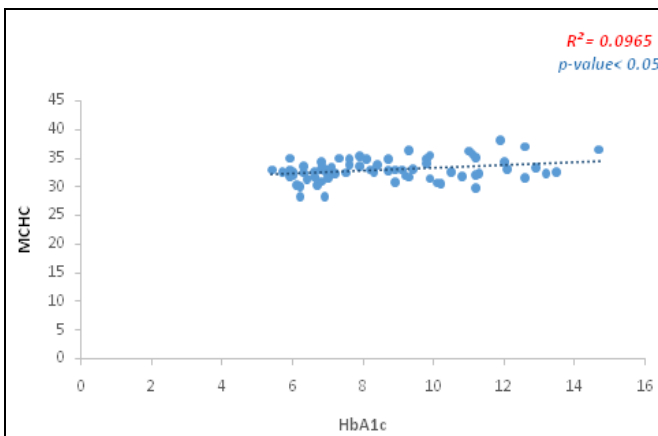


FIG. 2: SCATTER PLOT GRAPH OF MCHC AND HbA1c LEVELS IN THE TOTAL SAMPLE

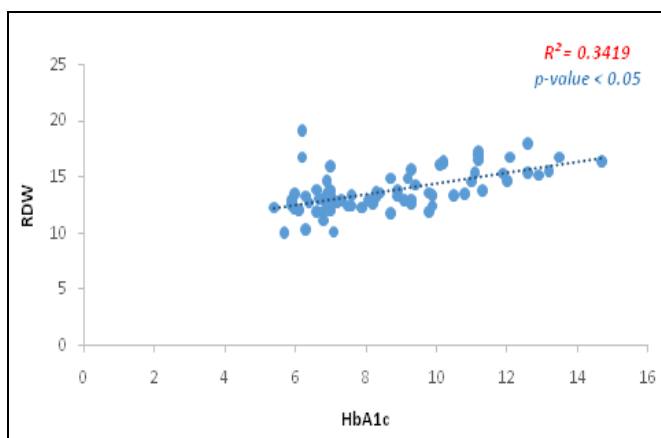


FIG. 3: SCATTER PLOT GRAPH OF RDW AND HbA1c LEVELS IN THE TOTAL SAMPLE

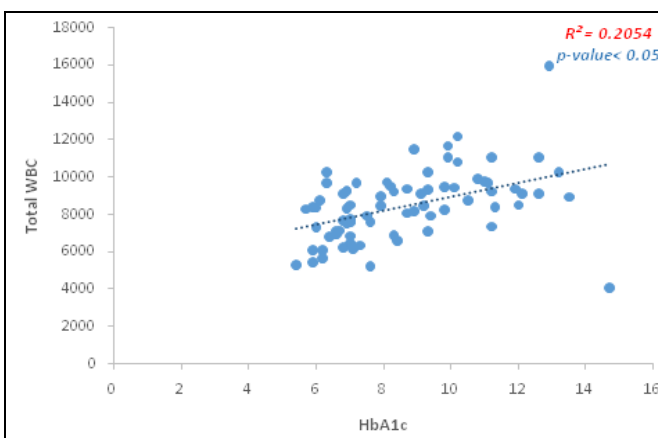


FIG. 4: SCATTER PLOT GRAPH OF WBC AND HbA1c LEVELS IN THE TOTAL SAMPLE

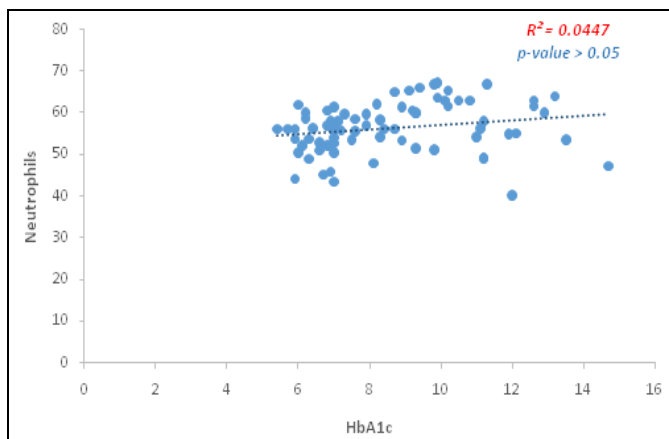


FIG. 5: SCATTER PLOT GRAPH OF NEUTROPHILS AND HbA1c LEVELS IN THE TOTAL SAMPLE

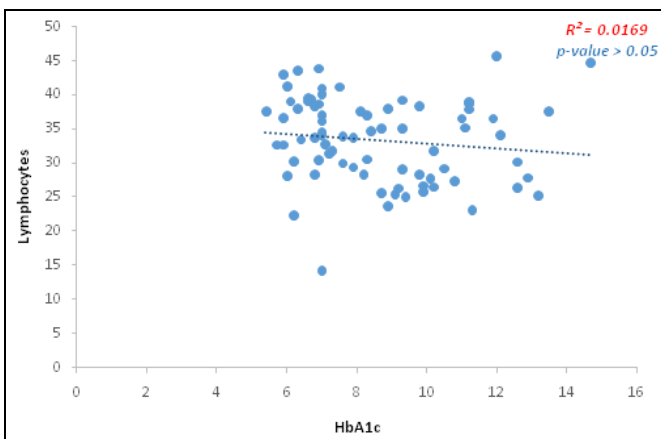


FIG. 6: SCATTER PLOT GRAPH OF LYMPHOCYTES AND HbA1c LEVELS IN THE TOTAL SAMPLE

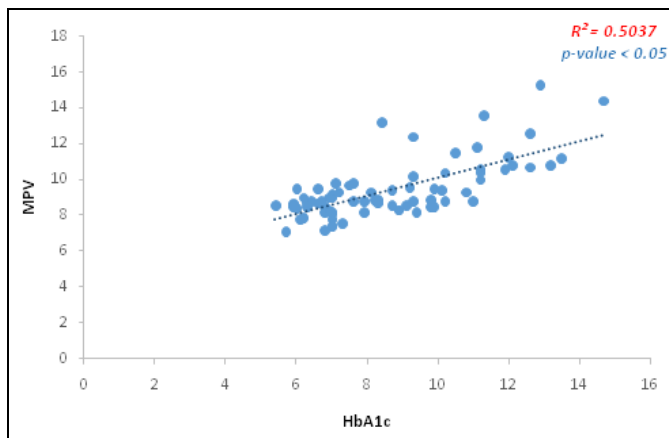


FIG. 7: SCATTER PLOT GRAPH OF MPV AND HbA1c LEVELS IN THE TOTAL SAMPLE

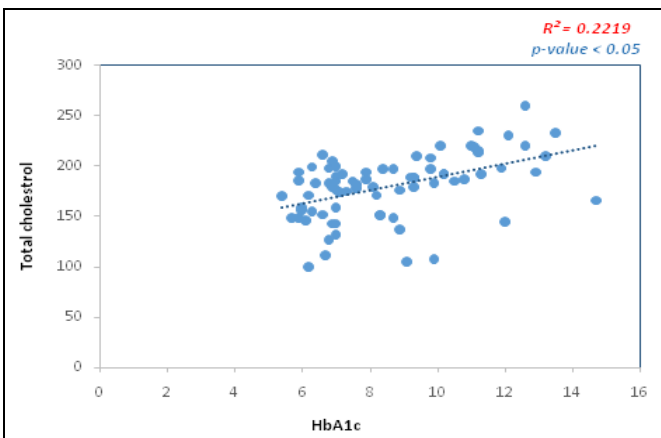


FIG. 8: SCATTER PLOT GRAPH OF TOTAL CHOLESTEROL AND HbA1c LEVELS IN THE TOTAL SAMPLE

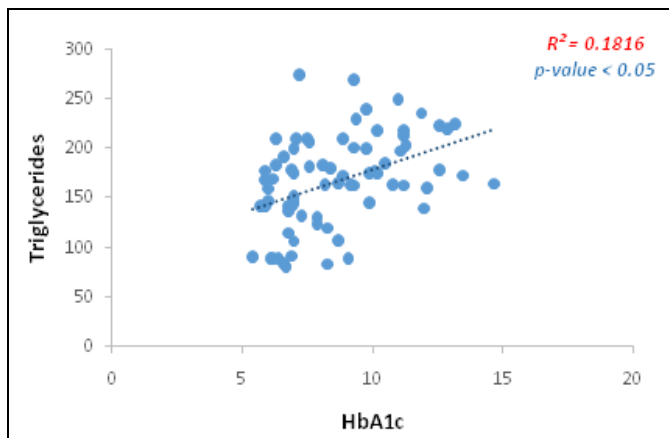


FIG. 9: SCATTER PLOT GRAPH OF TRIGLYCERIDES AND HbA1c LEVELS IN THE TOTAL SAMPLE

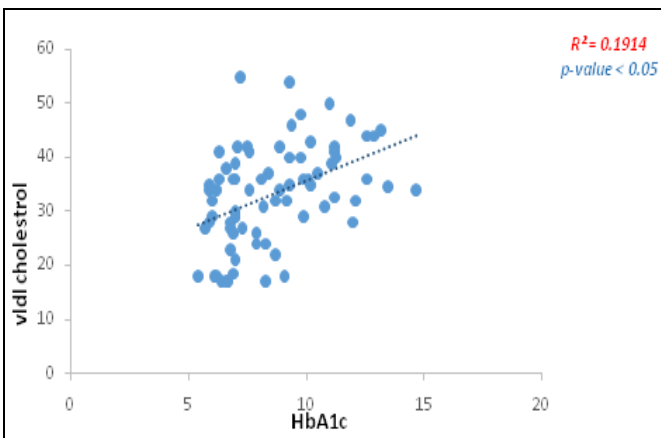


FIG. 10: SCATTER PLOT GRAPH OF VLDL AND HbA1c LEVELS IN THE TOTAL SAMPLE

DISCUSSION: Diabetes is associated with non-enzymatic glycosylation of hemoglobin²⁰. As the hyperglycemia persists, there are changes in the composition and morphology of erythrocytes, which affects the flow properties by altering erythrocyte deformability and aggregation²¹. The results of our study revealed that among the RBC indices MCH, MCHC and RDW showed a significant increase in mean (at p-value < 0.005)

within the normal range among the subjects with HbA1c% > 7. However MCH showed no correlation (r=0.185; p-value=0.107) with HbA1c%. MCHC, and RDW showed a significant positive correlation (r=0.310, r= 584; at p-value 0.000) with HbA1c%. RDW reflects the variability in red cell size, and vitamin B12 deficiency might also be associated with long-term metformin²² use which is important for the normal maturation of the red blood cells and

serves as a prognostic inflammatory marker for future complications.

Type 2 DM is a prothrombotic state due to increased platelet activity¹⁴. Platelet activity may be elevated by hyperglycemia and hyperlipidemia, insulin resistance, an inflammatory and oxidant state, and also with increased expression of glycoprotein receptors and growth factors²³. Our study results were similar to Kodiatte, Thomas Alex *et al.*²³ and Zuberi, B F *et al.*²⁴ We found that MPV significantly increased mean within normal in subjects with HbA1c > 7 (at p-value 0.000). MPV showed a significant positive correlation with HbA1c (r= 0.70 at p-value 0.00). A study by Aktas *et al.*, 2014²⁵ suggests that homocysteine, a determinant of atherosclerosis and a maker for inflammation²⁶ may be elevated in vitamin B12 deficiency. As MPV is also associated with inflammation, both homocysteine and MPV might be interrelated²⁷.

In the poorly controlled diabetes subjects, WBC, neutrophils showed a comparatively increased mean value in subjects with HbA1c% >7 than subjects with good glycemic control (9031.10 ± 1942 vs. 7467.17±1276.51) at p-value < 0.05. WBC showed a significant positive correlation (r=0.453 at p-value 0.00), whereas neutrophils did not show a significant positive correlation with HbA1c%. Lymphocytes were negatively correlated with HbA1c% and were low in the subjects with HbA1c %<7. Increased WBC is usually associated with a decrease in insulin sensitivity. A meta-analysis of prospective studies has reported that people in the top third of baseline total leucocyte counts have about a 40% (95% CI: 30–50%) increased risk of coronary heart disease (CHD) over the subsequent decade compared with those in the bottom third of the population^{28,29}.

In our study, we evaluated the relationship between total cholesterol, triglycerides, and VLDL with glycated hemoglobin. The lipid parameters TC, TG, VLDL showed an increment among the poorly controlled diabetes patients and showed a significant positive correlation (r = 0.471; 0.426; 0.437; at p-value 0.00) with HbA1c. Khan *et al.*, 2007³⁰, Meenu *et al.*, 2013³¹ studies showed similar results of correlation between TC, TG, VLDL, and HbA1c. Elevated triglycerides play a major role in increasing atherosclerotic cardio-

vascular risk³². All the lipid parameters under our study showed a significant increase and tended to the high borderline, which indicates increased cardiovascular risk among type 2 diabetes patients.

CONCLUSION: Our study demonstrated that HbA1c% was positively correlated with MCHC, RDW, MPV, WBC, TC, TG, and VLDL. Hyperlipidemia was also found to be persistent among patients with poor glycemic status, which is associated with a higher risk of cardiovascular-related mortality and morbidity. As a complete blood picture is simple and cost-effective, screening of hematological parameters and lipid parameters also should be done along with glycated hemoglobin to presume the risk of long-term vascular complications and to increase the quality of life in T2DM patients.

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CONFLICTS OF INTEREST: None

LIMITATIONS: A small sample size and limited resources were the limitations of our study. Further studies should be conducted with a large sample size.

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