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Correlation of HbA1c with Various Hematological Parameters in Patients of Type 2 Diabetes Mellitus in a Tertiary Health Care Centre in North India: A Comparative Cross-Sectional Study

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Abstract: *Introduction:* Diabetes has become one of the leading Global health Emergencies and is ranked among the top 10 causes of mortality worldwide. Diabetes mellitus causes a wide range of complications including micro and macrovascular complications like microangiopathy, retinopathy, nephropathy, neuropathy and diabetic foot. HbA1c is considered as the Standard of Care for monitoring type 2 Diabetes Mellitus. We aim to evaluate the common hematological parameters along with inflammatory parameters like neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in patients of type 2 diabetes mellitus. *Methods:* This was an observational laboratory based cross sectional study in which cases were included from 1st November 2023 to 31st December 2023. Two hundred and thirteen cases were divided into two groups (Diabetic and Non-Diabetic group) based on HbA1c levels and exclusion criteria. All cases were assessed for RBC count, TLC, DLC, Hb, RDW, Hct, NLR and PLR. Data was analysed using IBM SPSS v 29.0.2.0 (20) software and Microsoft Excel. A p value of less than 0.05 was considered statistically significant. *Results:* The mean age of diabetic and non-diabetic groups were 52.9 years and 50.6 years respectively ($p > 0.05$) with sex ratio of 1:2.1 and 1:1.7 respectively. There were significant differences in the means of the two groups for hematocrit levels, WBC count, neutrophil count, and NLR with p values of 0.001, 0.018, < 0.001 and < 0.001 respectively. There was no significant difference in the means for other parameters. *Conclusion:* The study results are consistent with the significant role of inflammation in etiopathogenesis of type 2 diabetes with significant increase in the levels of hematocrit, WBC count, neutrophils and NLR in patients with poor glycemic control. Furthermore, NLR being an easy and cost-effective tool along with its high association with microangiopathic complications, it needs to evaluated meticulously for assessing the prognosis in patients of type 2 diabetes mellitus.

Keywords: Diabetes Mellitus, Neutrophil-lymphocyte ratio, HbA1c, glycemic control, Inflammation

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INTRODUCTION

In 21st century, diabetes has become one of the leading health emergencies in the world and is ranked among the top 10 causes of mortality worldwide [1]. According to reports by World Health Organization (WHO), diabetes was linked to 1.6 million deaths in the year 2019 becoming the 9th most common cause of death globally [2]. The rapid socioeconomic development along with industrialisation and urbanisation in developing countries like India has led to a many fold increase in the disease burden [3]. The prevalence of diabetes in India has soared from 7.1% in 2009 to 8.9% in 2019 with trends suggesting that the number of patients with impaired glucose tolerance might reach up to 35.7 million by the year 2045 [1,4]. According to Indian Council of Medical Research (ICMR) in its India-Diabetes study, prevalence of diabetes was much higher in urban areas (11.2%) compared to rural areas (5.2%) with highest prevalence of diabetes seen in Chandigarh (13.6%) [5]. Diabetes mellitus is notorious for its wide range of complications including micro and macrovascular complications and even death. The most common among these are microangiopathy, retinopathy, nephropathy, neuropathy and diabetic foot [6]. Diabetes also makes the patients vulnerable to lot many infections, myopathyies and osteoporosis. It is a well-known fact that atherosclerosis is in inflammatory disorder with all the cellular elements of blood such as leukocytes, erythrocytes and platelets playing a major pathogenic role [7]. Various

studies across the literature have linked diabetes and its complications to leukocyte, erythrocyte and platelets dysfunction and their altered levels [8, 9]. Increased white blood cell (WBC) count had an association with higher cardiovascular death in patients with type 2 diabetes mellitus and coronary artery disease patients showed an increase in neutrophil counts in these patients [10-12]. Diabetic patients with poor glycemic control had shown a higher red cell distribution width (RDW) in the study conducted by Nada *et al*., [13]. Chen *et al*., reported that there was a significant increase in Mean Platelet volume (MPV) but platelet count and platelet distribution width (PDW) were not increased in T2DM [14]. Glycated hemoglobin i.e. HbA1c provides evidence about an individual's average blood glucose levels for the period of previous three months and is now considered as the standard of care for testing and monitoring type 2 diabetes mellitus [15]. In this study we aim to evaluate the common hematological parameters like RBC count, haemoglobin, haematocrit, total leukocyte count (TLC), differential leukocyte count (DLC), red cell distribution width (RDW), red cell indices along with common inflammatory parameters like neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in patients with glycated hemoglobin (HbA1c) > 6.5 %.

MATERIALS AND METHODS

This was an observational, laboratory based cross sectional study done in the Civil Hospital, Ambala Cantt, Haryana. The study was carried out for a period of two months, starting from 1st November 2023 to 31st December 2023. Two groups based on HbA1c levels who were subjected to complete blood cell count tests based on inclusion and exclusion criteria were included in the study. Group 1 (diabetic group) comprised of 133 cases and 80 cases were included in group 2 (non-diabetic group). All samples with HbA1c levels > 6.5 % were taken in group A (diabetic group) whereas samples with HbA1c levels < 5.6 % were taken in group B (non-diabetic group). Patients below 18 years of age, smokers, pregnant females and first time diagnosed cases of diabetes mellitus were excluded from the present study. Patients having anemia of any cause, hemoglobinopathies or coagulation disorder or patients suffering from any acute or chronic infectious disorders were also excluded. Patients with chronic disorders like chronic liver disease, renal failure, any autoimmune disorder or malignancy did not form the part of this study. Blood samples were collected after a night time fasting of at least 12 hours in EDTA (ethylene diamine tetracetic acid) vial and testing of samples was carried at the hospital laboratory, Civil Hospital, Ambala Cantt. HbA1c was analysed using Finecare Wondfo HC-B014E PLUS immunoassay analyser. RBC count, TLC, Hb, RDW, Hct, red blood cell indices were analysed using Sysmex XP100 cell counter analyser and DLC was done manually. NLR and PLR were calculated using the following formulas.

NLR = absolute neutrophil count /absolute lymphocyte count; PLR = absolute platelet count / absolute lymphocyte count

Data was analysed using IBM SPSS v 29.0.2.0 (20) software and Microsoft Excel. Student's t test was used for comparing means of the two groups and Levene's test for equality of variance. A p value of less than 0.05 was considered statistically significant. Graphical interpretations of data for easy comparison of the two groups were also provided.

RESULTS

The study population comprised of 233 cases, out of which 133 cases were a part of the diabetic group (group 1) and 80 cases were included in the non-diabetic group (group 2). The mean age of group 1 and group 2 were 52.9 years and 50.6 years respectively with a mean difference of 2.3 years (p value > 0.05). In group 1, 43 cases were male and 90 were female whereas in group 2, 29 cases were male and 51 were female with the sex ratio of 1:2.1 and 1:1.7 respectively. Comparison of means and other significant data of all the CBC parameters included in the study between the diabetic and non-diabetic groups are given in Figure 1. Mean differences of the two groups along with their corresponding p values are given in Table 1. For parameters like hemoglobin, RBC count, lymphocyte count, platelet-lymphocyte ratio, red cell distribution width, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, no significant difference was noted between the groups with p values >0.05 for parameters. However, the results revealed that there were significant differences in the means of the two groups for hematocrit levels, WBC count, neutrophil count, and NLR with p values of 0.001, 0.018, <0.001 and <0.001 respectively. Line graphs drawing a comparison between the two groups for hematocrit, TLC and NLR are given in Figure 2, 3 and 4 respectively.

	GROUP 1					2					Total
	N	Mean	Std. Deviation	Maximum	Minimum	N	Mean	Std. Deviation	Maximum	Minimum	N
Age	133	52.99	13.450	85	19	80	50.66	15.240	79	19	213
HbA1c	133	9.457	1.9403	14.5	6.5	80	4.878	.3341	5.6	4.0	213
Hb	133	13.927	1.4725	18.0	12.0	80	13.169	1.1238	16.2	12.0	213
Hct	133	41.481	3.1495	52.9	35.3	80	40.529	1.8890	44.8	37.3	213
RBC	133	4.8159	.47063	5.80	3.70	80	4.4711	.49447	5.67	3.85	213
TLC	133	8385.71	2277.763	16200	3300	80	6353.74	1535.243	11200	4100	213
N	133	61.76	8.938	84	41	80	57.72	5.835	71	44	213
L	133	29.99	8.555	50	7	80	33.69	6.324	53	20	213
M	133	4.98	2.060	13	1	80	5.92	1.652	11	2	213
RDW	133	13.574	.9816	17.2	11.3	80	13.532	.8141	15.2	11.9	213
MCV	133	86.938	4.8905	107.0	76.0	80	87.049	3.9333	96.3	77.7	213
MCH	133	29.110	1.9723	35.0	24.0	80	28.565	1.7147	33.1	24.5	213
MCHC	133	33.258	1.3950	36.4	30.0	80	32.190	1.5857	36.0	28.7	213
NLR	133	2.399450	1.4171465	11.8571	.8200	80	1.810656	.5588111	3.5500	1.0000	213
PLR	133	107.401462	61.1151232	543.3071	33.7999	80	139.377896	63.1090439	363.9535	39.2716	213
PLT	133	237.50	83.842	518	89	80	272.86	87.592	445	121	213

Figure 1: Comparison of means of various parameters between two groups

Hb- hemoglobin(g/dl)); Hct- hematocrit(%); RBC-red blood cells(/mcL); TLC- total leukocyte count(/mcL); Nneutrophils(%); L-lymphocyte(%); M-monocyte(%); RDW-red cell distribution width(%); MCV-mean corpuscular volume(fl); MCH-mean corpuscular hemoglobin(pg); MCHC; mean corpuscular hemoglobin concentration(g/dl); NLR-neutrophil lymphocyte ratio; PLR-platelet lymphocyte ratio; PLT-platelets(/mcL).

Table 1: Mean difference and p values between groups of various parameters

	Hb	Hct	RBC	TLC	RDW	MCV	MCH	MCHC	PMN	NLR	PLR	PLT
Mean difference	.75	.95	.34	2031.9	.04	-.11	.54	1.06	4.03	.588	-31.97	-35.3
p value	.053	.001	.964	.018	.593	.119	.194	.156	<.001	<.001	.068	.45

Hb- hemoglobin(g/dl)); Hct- hematocrit(%); RBC-red blood cell(/mcL); TLC- total leukocyte count(/mcL); RDW-red cell distribution width(%); MCV-mean corpuscular volume(fl); MCH-mean corpuscular hemoglobin(pg); MCHC; mean corpuscular hemoglobin concentration(g/dl); PMN-neutrophils(%); NLR-neutrophil lymphocyte ratio; PLR-platelet lymphocyte ratio; PLT-platelets(/mcL).

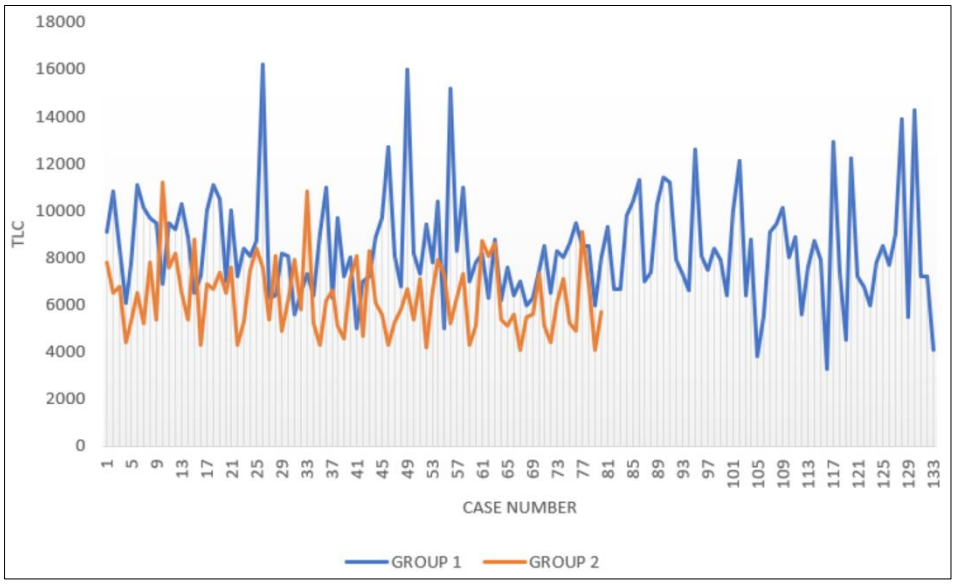

Figure 2: Graphical representation drawing a comparison of NLR between two groups

NLR- neutrophil-lymphocyte ratio

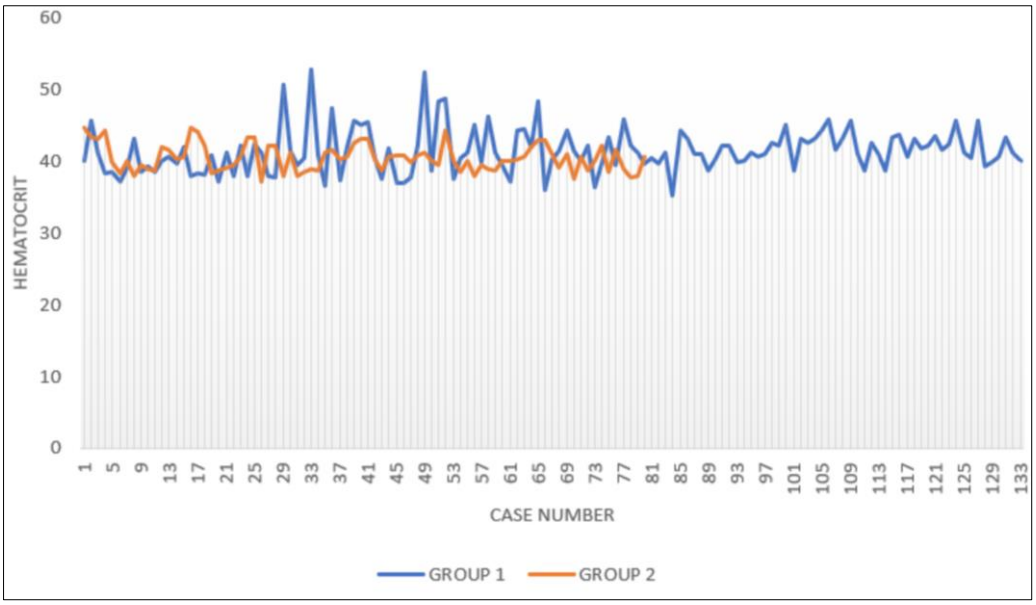

Figure 3: Graphical representation drawing a comparison of haematocrit (%) between two groups

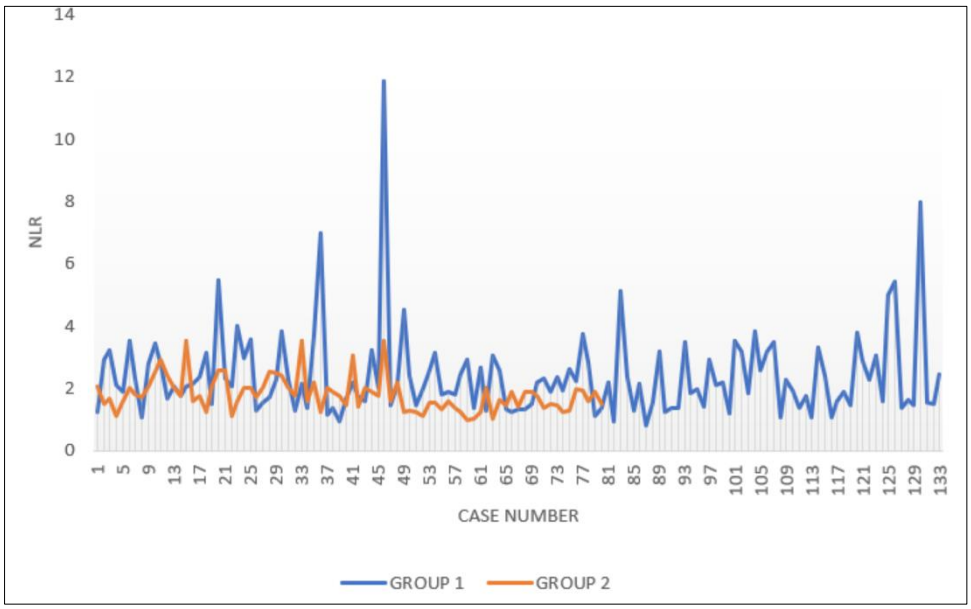

Figure 4: Graphical representation drawing a comparison of total leukocyte count between two groups

TLC- total leukocyte count/mcL

DISCUSSION

World Health Organization (WHO) describes diabetes mellitus as a chronic metabolic disorder with persistently raised blood glucose levels leading to systemic damage over time. Pathogenesis of diabetes mellitus is complex and involves multiple factors with each factor playing a significant role. The interrelationship of factors like genetic susceptibility, unhealthy lifestyle, inflammation, adipokines, free fatty acids, glucotoxicity and insulin resistance are involved in the etiopathogenesis of diabetes mellitus. In the present study we saw significant increase in hematocrit, white blood cell (WBC) count, neutrophils and neutrophil-lymphocyte ratio in diabetic groups as compared to non-diabetic patients. All the deranged parameters are linked to inflammatory processes and their effects. WBC count and a novel marker, NLR are predictive markers of inflammation and have been directly linked to rise of tumor necrosis factor-α (TNF-α), C-reactive protein (CRP) and Interleukin 1 and 6 in various studies across literature. A dominant role of NLR in causing microangiopathic damage in patients with long standing diabetes has also been seen in multiple studies conducted on diabetic patients across

various countries [16]. Shiny *et al*., and Lou *et al*., in their studies revealed that an increased NLR had a strong association with poor glycemic control and insulin resistance in patients of type 2 diabetes mellitus [17, 18]. Also, studies which were conducted on diabetes related micro vascular complications revealed that NLR is a reliable predictive marker of early-stage diabetic nephropathy, foot ulcer and retinopathy [19-21]. Moreover, increase in NLR is also linked to major cardiovascular events in acute coronary syndrome patients as well as coronary artery disease patients and is also associated with increased intima-media thickness of carotid artieries in these patients [22, 23]. This study too had similar findings and revealed that patients with poor glycemic control i.e., with HbA1c levels of greater than 6.5 %, have a greater NLR as compared to patients with normal glycemic control. Among different inflammatory markers, measuring white blood cells (WBC) along with NLR, is simple and can be done in any clinical setting without much expenditure on tests. Findings of our study also showed that WBC count was significantly higher in diabetic group compared to control group. One plausible explanation to increased WBC count is that both a higher WBC count and insulin resistance reflect an underlying activation of the immune system. IL-6, a potent WBC differentiation marker has been linked to insulin resistance and obesity [24, 25]. Also, the hematocrit levels of diabetic group were significantly higher as compared to the control group in our study. A significant number of researchers across literature have showed that the blood viscosity was altered in patients with diabetes [26, 27]. As the osmolarity of the blood increases due to increased blood sugar levels, the capillary permeability also increases, and thus increasing the hematocrit and subsequently the blood viscosity [28]. Also, another possible explanation is that increased microvascular permeability may lead to reduced plasma volume and thus increased hematocrit levels [29]. This has also been associated with slowed retinal circulation further leading to retinopathy in diabetic patients. Barnes *et al*., in their study reported that both hematocrit and blood viscosity decreased after a good diabetic control was achieved by the patients with previously high blood sugar levels [30]. Thus, increased levels of Hematocrit should caution the clinician about the microangiopathic damage increased viscosity could cause and the clinician should plan the treatment accordingly. Few limitations of our study were that it was a cross-sectional design in which nothing about the causality of the variables could be assessed. Secondly, analyses were based on a one-time measurement of CBC parameters and could not reflect the relation of HbA1c with CBC parameters over time. Thirdly, systemic and microangiopathic complications could not be assessed in the patients for CBC correlation.

NLR being a novel and a promising inflammatory marker requires more extensive analysis and should be involved in the list of primary investigations in patients of diabetes mellitus in coming years. NLR and WBC count, due to their cost effectiveness, easy assessment and due to their strong association to atherosclerotic microvascular effects, they should carefully and cautiously be evaluated while assessing and treating patients of type 2 diabetes mellitus.

CONCLUSION

Our study results were consistent with the significant role of inflammation in etiopathogenesis of type 2 diabetes. The study revealed that there was significant increase in the levels of hematocrit, WBC count, neutrophils and NLR in patients with poor glycemic control as compared to patients with normal HbA1c levels. All these parameters that were deranged in our study can lead to microangiopathic complications in patients of long-standing diabetes mellitus. Furthermore, NLR is an easy and cost-effective tool in assessing the immunological response and for assessing the prognosis as well as predicting the chances of complications in patients of type 2 diabetes mellitus. Therefore, NLR should form a part of the primary investigations evaluated in patients of diabetes mellitus.

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