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"Correlation of platelet indices with HbA1c in non diabetic and diabetic patients with or without complications – A Case Control Study"

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Abstract

Background: A persistent metabolic syndrome is diabetes mellitus marked mostly by chronic hyperglycemia. Recently,it is considered as state of prothrombosis,characterized by abnormalities in platelet function which has been recognized as a component of the metabolic syndrome. Aims are made to discover and demonstrate the utility of various blood tests, including platelet indices for the early diagnosis of diabetic problems. Increased platelet activity has a role in development of diabetic complications, thus platelet volume indices are considered as potential biomarkers in diabetics who have vascular issues.

Methods : 356 people with diabetes and 201 non-diabetics participated in the study. To perform a complete hemogram, a Sysmex KX-21 automatic blood counter and a Benesphera H51 coulter were used. From the records of the Central Clinical Laboratory, the HbA1c level was assessed using the immunoturbidometric inhibition technique. The statistical analysis was carried out using SPSS version 17 (Statistical Package for the Social Sciences) (Chicago, IL). Student's t-test was used to determine if there was a significant difference in HbA1c, PC, MPV and PDW. Statistics were judged significant at P 0.05.

Results : In our study, MPV and PDW was statistically significant in diabetic patients with complications (P<0.0001 and P=0.0001respectively). A positive correlation was seen between MPV and HbA1c. While MPV was statistically significant in diabetic retinopathy, nephropathy, and diabetic foot, PDW was statistically significant in diabetic retinopathy and nephropathy.

Conclusion : An effective predictor of diabetic cardiovascular issues would be MPV. Additionally, we found a direct correlation between an increase in MPV and a rise in HbA1c levels. Therefore, we propose

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that MPV can be used as a simple and low-cost method to monitor the onset and control of Diabetes Mellitus and its cardio-vascular effects.

Keywords : Diabetics , platelet indices, complications, HbA1c

INTRODUCTION

In the modern world, diabetes mellitus is viewed as a pandemic [1]. A metabolic syndrome characterised by vascular issues and prolonged hyperglycemia. The detrimental lifestyle changes with excess calorie intake and decreased physical activity are the main causes of the rising incidence of DM, which will be more than double in the next 15 years [2].India has the greatest prevalence of diabetic patients.

The syndrome of dysmetabolism is a cluster of phenotypes that is strongly associated with a higher risk for cardiovascular disease and is prevalent among people with diabetes type 2 and impaired glucose tolerance. A major contributing factor to this syndrome is insulin resistance. Obesity, hypertension, impaired lipid profile (low High Density Lipoprotein and oxidised Low Density Lipoproteins with high triglyceride), and endothelial impairment are additional contributing factors (microalbuminuria)[3].

The metabolic syndrome now includes a prothrombotic condition, which is defined by impairments in platelet function and elevated blood levels of fibrinogen, plasminogen activator inhibitor(PAI-1), and C-reactive protein(CRP). Retinopathy, nephropathy, and neuropathy are examples of microvascular diseases that significantly enhance morbidity in diabetes mellitus. However, macrovascular complications are the main factor in morbidity and mortality in DM. Over 75% of diabetic individuals pass away from heart problems. Impaired insulin sensitivity, Prediabetes, and overt type are all significantly more prevalent in person with type 2 DM compared to non-diabetics , as are heart problems, peripheral arterial disease, and higher increased risks for stroke. Additionally, after cardiac procedures and in acute coronary syndromes, diabetes impairs both early and late outcomes. Both types of diabetes are characterised by premature, accelerated macrovascular disease. Recent epidemiological studies show that both types of diabetes significantly increase the risk of cardiovascular death and stroke, and that both of these problems can develop in children[5].

In atherothrombotic illness, platelet function is crucial for pathophysiology, and type 1 and type 2 diabetes both have substantial evidence for platelet dysfunction and hyperreactive platelets. The development of diabetic angiopathy may be largely attributed to platelets, which, in cooperation with the vascular endothelium, leukocytes, and coagulation, are thought to be important contributors. The pathophysiology of macro angiopathy obviously involves platelet dysfunction, but the pathogenesis of micro angiopathy is less evident. Even diabetic disease in its early phases diabetic disease in its early phases, the state of metabolism that goes along with diabetes mellitus may affect the function of platelets and endothelial cells. It is questionable, therefore, whether anti-diabetic medication and better metabolic management will bring back the observed platelet hyperactivity in DM. There are very few research on how acute hyperglycemia affects DM patients' platelet function. It is underlined that the elevated platelet activity contributes to the vascular consequences of this illness. Hefty platelets have more hemostatic activity and can increase the risk of coronary blood clot, which can result in myocardial infarction. People who have extensive histories of diabetes type 2 and poor glycemic control to a greater extent develop diabetic micro-vascular complications. Diabetes has been linked to increased platelet activity, as seen by a rise in platelet glycoprotein IIb/IIIa, Gp Ib-IX, and Gp Ia/IIIa[6].

Platelet Volume Indices are being evaluated with the increasing availability of blood cell analyzers. Most frequently employed measurement of thrombocyte size is also a possible indicator of blood platelet activity. This new risk factor for atherosclerosis is now emerging[7-10]. During routine haematological

examination, patients with bigger platelets can be easily detected and may benefit from the treatment for anticipating potential acute episodes. HbA1c levels can be used to calculate the average blood sugar level. It is a crucial indicator of how well diabetes has been managed during the past three months.

According to studies on the function of glycated haemoglobin, it represents blood glucose levels on average over a long period of time and is uninfluenced by short-lived fluctuations in sugar amounts. The measurement of glycated haemoglobin levels is a practically appropriate examination for evaluating how well diabetes is managed in order to prevent its complications. This test is sensitive and specific for identifying early diabetics, at-risk persons, and people with undiagnosed diabetes. Poor metabolic management raises the chance of complications such nephropathy and retinopathy. HbA1c, which serves as a marker for the average blood sugar level, forecasts the start of problems in diabetics.

Materials and Methods

A tertiary care hospital and research facility in Maharashtra served as the study's location. 201 non-diabetics without complications and 356 diabetic patients participated in the study. Over the course of a year, all patients and controls were selected from the outpatient medicine department. Before the study began, approval from the institutional ethical committee was acquired. The patients' written and informed permission was obtained. Patients with diabetes who were given diagnoses in accordance with the American Diabetes Association were included[11]. According to their medical records, the people in the control group were those without DM.

The study excluded participants with anaemia (13gm% of male subjects and 12gm% of female subjects), patients with inflammatory diseases (rheumatoid arthritis, S.L.E.) , cancer, chronic renal failure, cyanotic heart disease, thrombocytopenia, and hypo- or hyperthyroidism and people with diabetes taking antiplatelet medications like aspirin and clopidogrel.

Each individual, whether they had diabetes or not, got a thorough clinical evaluation that paid particular attention to potential micro- and macro-vascular problems. A clean puncture was used to obtain a blood sample from the antecubital vein while following all aseptic procedures to prevent bubbles and froth. A total of three blood samples, 2 ml each in an EDTA, fluoride, and plain bulb, were taken. Sysmex KX-21 automatic blood counter and H51 Benesphera coulter from an EDTA bulb were used to complete a full hemogram. Additionally recorded were Hb, platelet count, MPV and PDW. The glucose oxidase technique was used to determine the plasma glucose levels. Using the immunoturbidometric inhibition technique, the HbA1c level was assessed from the Central Clinical Laboratory's records..

Additionally, diabetic patients were assessed for several macrovascular problems like CAD, PAD, and diabetic foot as well as microvascular issues like diabetic retinopathy, neuropathy, and nephropathy. Clinical symptoms and 2D echocardiography (ECHO) patient observations were used to determine the presence of CAD. Clinical symptoms, the capacity to walk for long distances, and a lower limb Doppler ECHO scan had all been used to identify PAD. Five National Diabetes Education Program criteria—sensory neuropathy, absence of pedal pulses, foot deformity, present or previous foot ulcer, and history of foot amputation—were used to identify diabetic foot[12]. The discovery of at least two microaneurysms and/or retinal damage in the records served as the basis for the diagnosis of retinopathy. For the conventional diagnosis of diabetic nephropathy, the quantitative urine albumin/creatinine ratio in the morning spot urine samples, increased BUN, and elevated creatinine were employed. Diabetic neuropathy was diagnosed using nerve conduction velocity (NCV)test and electromyography (EMG).

Statistical Package for the Social Sciences (SPSS) version 17 was used to conduct the statistical analysis (Chicago, IL). According to problems, the Student's t-test was used to determine if there was a significant

difference in FBS, HbA1c, PC, MPV and PDW while the ANOVA test determined whether there was a significant difference in PC, MPV and PDW between the three groups. Standard deviation was used to express the data as mean. Statistics were judged significant at P 0.05.

RESULTS

Two groups – **(Grp A)** 201 controls (122 males, 79 females) and **(Grp B)** 356 diabetic patients (226 males, 130 females) in total were chosen for the study. Majority of diabetics were males particularly between the ages of 45 and 65 . In both the groups, HbA1c and platelet indices were compared.

Three categories on the basis of HbA1C value were made for the study **(Diabetics, Pre-Diabetics and Normal)**.(FIG 1) HbA1c was slightly higher in patients of diabetes with predominantly microvascular complications(117 out of 356 cases) as compared to macro-vascular or without complications case along with controls.(FIG 2,3A & 3B)

According to the study, MPV was markedly higher in diabetic patients with complications (P<0.0001) than in diabetics without complications or in the non-diabetic group. Between diabetics with complications, diabetics without complications, and non-diabetic group, there was a statistically significant difference in PDW (P 0.0001). (FIG 4)

The investigation confirmed that elevated FBS levels and poor glycemic control increased the risk of diabetes complications. It was demonstrated how platelet indices relate to different diabetic complications.

We discovered a statistically significant relationship between MPV and diabetes retinopathy, nephropathy, and diabetic foot (P = 0.0000, 0.0036, and 0.0935, respectively). MPV is elevated but not statistically significant in the remaining problems. (FIG 5) PDW increased statistically in diabetic retinopathy and nephropathy (P = 0.0000,0.0028 respectively). (FIG 6) No statistically significant change was seen in platelet count in diabetics. (FIG 7, 8)

With increasing DM duration, increased MPV was discovered to be significant There was, however, no statistically significant relationship between platelet count, PDW.

A positive correlation was seen between MPV and HbA1c while no positive correlation was seen with PC & PDW with HbA1c. (FIG 9,10,11)

DISCUSSION

Our study shows that in Diabetics, platelet count were lower than those of the control group. However, there was no statistically significant relationship between platelet count and diabetic sequelae. Increasing platelet reactivity in diabetes patients is thought to be a result of insulin resistance and hyperglycemia, two key contributing variables. A well-known component that contributes to the prothrombotic state in diabetics and results in enhanced coagulation, poor fibrinolysis, and endothelial dysfunction is platelet hyper-reactivity. In the pathophysiology of the thrombotic events leading to diabetes complications, these hyperactive platelets play a crucial role[14].

As a measure for measuring platelet size and a potential biomarker of platelet reactivity, MPV is used. Larger platelets have been found to be more reactive than smaller ones. According to our research, significantly more MPV was present in diabetics who have complication than in diabetics who do not have complications or in the non-diabetic group. Increased MPV is linked to poor glycometabolic management and manifests itself in a wide range of problems, including retinopathy, nephropathy, CAD, and diabetic foot. We found an association between MPV and retinopathy, nephropathy, and diabetic foot that was statistically significant; investigations by Dindar et al. and Ates et al. also showed higher values in this regard[13-16]. However, in investigations conducted by Demirtunc et al., MPV was not substantially different in individuals with these problems[17]. Additionally, diabetic retinopathy's retinal

neovascularization was linked to MPV. MPV was found to be higher in the remaining issues, although it was not statistically important. There was an positive correlation between MPV and HbA1c in patients of diabetes with complications in the scatter diagram.

The diversity in platelet size can be directly measured by PDW, and high values indicate an increased production of bigger reticulated platelets[15]. Between group of people without diabetes, people with diabetes who have complications, and diabetics with no complications, PDW showed a statistically significant difference. PDW increased statistically in diabetic retinopathy and nephropathy and was also higher in complications including CAD and diabetic foot, although not statistically significant. While no positive correlation was seen with PDW with HbA1c in diabetics with complication in the scatter diagram.

Numerous investigations revealed a favourable connection between HbA1c and platelet indices. Some investigations, however, failed to find any connection between HbA1c and platelet indices. It has been suggested that the rise in MPV may be related to diabetic patient's elevated blood sugar levels, which produce osmotic swelling and shorter platelet life spans. Alternately, this might imply that platelet activation and glycemic control are linked.

The cardiovascular comorbidities such as hypertension, albuminuria, obesity, smoking, and dyslipidemia, as well as platelet number and reactivity, all contribute to the development of diabetes and its impact on platelet indices. Thus, it demonstrates that there are numerous more elements that could eventually account for the thrombotic risk of diabetics. Diabetes causes a dysregulated signalling system, which causes platelets to become more active and aggregate more frequently. This aids in the development of thrombus and microcapillary embolization. Local vascular lesions, such the neovascularization of the lens in diabetic retinopathy, advance more quickly as a result of the production of constrictive, oxidative, and mitogenic chemicals including platelet-derived growth factor and vascular endothelial growth factor.

The literature search turned up very little information on these more recent biomarkers. However, additional prospective studies with larger sample sizes are needed to determine the usefulness of these markers to forecast the burden of the diabetic disease, taking into account all the compounding risk factors, particularly to forecast the influence of platelet indices on diabetic complications.

CONCLUSION

Platelet mean volume, reactivity and aggregability rise in diabetes mellitus. The larger platelets may contribute to the increased risk of vascular problems and atherosclerosis that come with diabetes mellitus. In light of this, MPV would be a helpful predictive predictor of diabetic cardiovascular problems. Additionally, we discovered that an increase in MPV was directly linked to an increase in HbA1c concentration. But more research is required to determine if vascular issues are the root of or a consequence of elevated MPV. Therefore, we suggest that MPV be utilised as a straightforward and affordable technique to track the development and management of Diabetes Mellitus and associated cardio-vascular consequences.

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TABLE 1. FREQ HbA1C_Cat

HBA1C_CAT	Frequency	Percent	% CI
Diabetic (>=6.5%)	51	63.02%	58.93-66.92%
Normal (<5.7%)	110	19.75%	16.65-23.26%
Pre-Diabetic (5.7-6.4%)	96	17.24%	14.33-20.59%
Total	557	100.00%	

TABLE 2.TABLES HbA1C_Cat Complications_Cat

	Complications_Cat		
HBA1C_CAT	**with complications**	**without complications**	**Total**
Diabetic	183	168	351
Normal	1	0	1
Pre-Diabetic	1	3	4
TOTAL	185	171	356

TABLE 3 A. TABLES HbA1C_Cat Complications

	Complications				
HBA1C_CAT	**macro**	**micro**	**mixed**	**normal**	**Total**
Diabetic	58	117	8	168	351
Normal	1	0	0	0	1
Pre-Diabetic	1	0	0	3	4
TOTAL	60	117	8	171	356

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FREQ Complications

COMPLICATIONS	Frequency	Percent	95%CI
Macro	60	16.85%	13.32% - 21.09%
Micro	117	32.87%	28.19% - 37.90%
Mixed	8	2.25%	1.14% - 4.37%
Normal	171	48.03%	42.89% - 53.22%
Total	356	100.00%	

TABLE 3B. HbA1c compared to individual diabetic complications

Complications		HbA1c			
		Mean	SD	u value	p value
Retinopathy	Present	9.6458	2.6307	7.5707	0.0059
	Absent	8.6396	1.9105		
Nephropathy	Present	9.0023	2.3197	0.0442	0.8334
	Absent	8.8565	2.1167		
Neuropathy	Present	9.0821	2.4607	0.1010	0.7507

	Absent	8.8564	2.1130		
CAD	Present	8.8468	2.4067	0.5323	0.4657
	Absent	8.8799	2.0831		
PVD	Present	7.9500	1.7678	0.4022	0.5259
	Absent	8.8794	2.1422		
Diabetic Foot	Present	8.5700	2.4667	0.8206	0.3650
	Absent	8.8829	2.1326		

TABLE 4. Platelet indices compared with or without complications in diabetic patients

Complications	**PC**		**MPV**		**PDW**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
With complications	2.5930	0.9032	10.7568	2.2698	12.8449	3.0346
Without complications	2.4692	0.7219	10.0509	1.6245	14.9333	3.7347

TABLE 5. Complications of Diabetes in relation to MPV

Complications		**MPV**			
		Mean	**SD**	**u value**	**p value**
Retinopathy	Present	12.1675	2.8594	26.9528	0.0000
	Absent	14.3590	3.5753		

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Nephropathy	Present	12.4558	3.3478	8.4549	0.0036
	Absent	14.0393	3.5299		
Neuropathy	Present	14.1786	3.1631	0.6692	0.4133
	Absent	13.8198	3.5752		
CAD	Present	13.8419	3.0617	0.1504	0.6982
	Absent	13.8493	3.6396		
PVD	Present	11.4000	2.6870	1.2620	0.2613
	Absent	13.8619	3.5441		
Diabetic Foot	Present	12.0500	2.5448	2.8133	0.0935
	Absent	13.9000	3.5556		

TABLE 6. Complications of Diabetes in relation to PDW

Complications		**PDW**			
		Mean	**SD**	**u value**	**p value**
Retinopathy	Present	11.4783	2.5232	22.2285	0.0000
	Absent	10.0952	1.7118		
Nephropathy	Present	10.5047	1.8259	0.4409	0.0028
	Absent	10.4058	2.0416		
Neuropathy	Present	11.2679	1.7010	10.0082	0.816
	Absent	10.3451	2.0249		
CAD	Present	9.7048	1.5444	8.9279	0.0828
	Absent	10.5680	2.0714		

PVD	Present	9.9500	1.4849	0.0879	0.7669
	Absent	10.4203	2.0185		
Diabetic Foot	Present	11.0300	1.9178	1.3454	0.2461
	Absent	10.4000	2.0172		

TABLE 7. TABLES PC_Cat Complications

	Complications				
PC_CAT	**macro**	**micro**	**mixed**	**normal**	**Total**
<1.5	4	8	0	8	20
>4.5	2	2	0	1	5
1.5-4.5	46	91	2	142	281
TOTAL	52	101	2	151	306

TABLE 8. Complications of Diabetes in relation to Platelet Count

Complications		**PC**			
		Mean	**SD**	**u value**	**p value**
Retinopathy	Present	2.4677	0.8003	0.1581	0.6909
	Absent	2.5536	0.8293		
Nephropathy	Present	2.4595	1.1303	2.2917	0.1301
	Absent	2.5437	0.7724		
Neuropathy	Present	2.4964	0.6769	0.0178	0.8939

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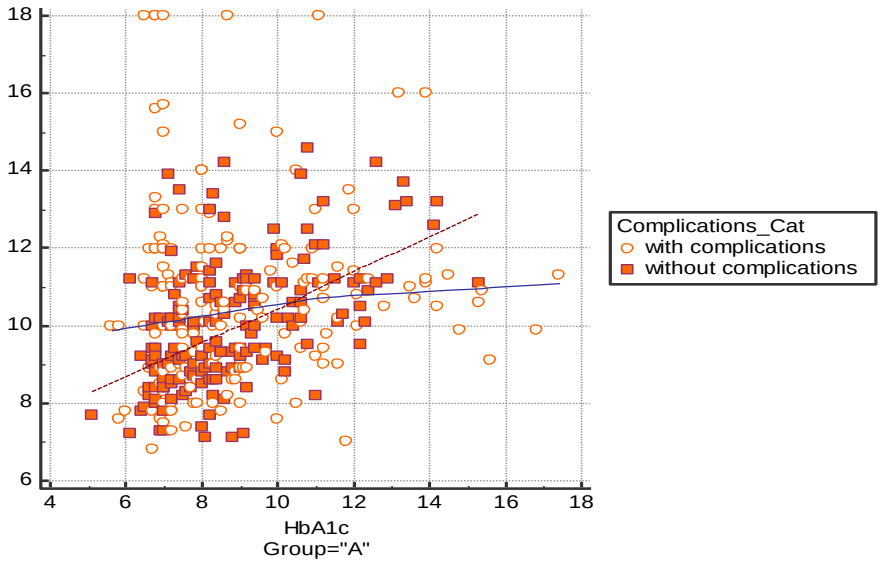
	Absent	2.5367	0.8344		
CAD	Present	2.7990	0.8723	8.9389	0.0028
	Absent	2.4776	0.8018		
PVD	Present	3.0000	1.1314	0.4634	0.4960
	Absent	2.5309	0.8217		
Diabetic Foot	Present	2.5500	0.6587	0.0323	0.8573
	Absent	2.5331	0.8274		

Correlation FIG 9.

Variable Y	MPV
Variable X	HbA1c
Filter	Group="A"

Sample size	356
Correlation coefficient r	0.2181
Significance level	P<0.0001

Scatter diagram



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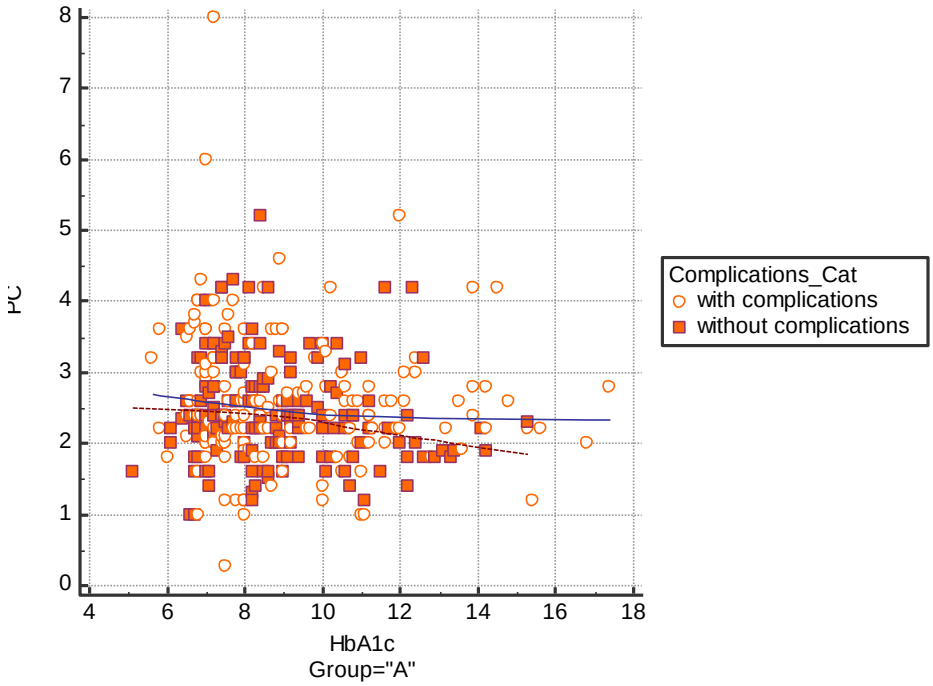
Correlation FIG 10.

Variable Y	PC
Variable X	HbA1c
Filter	Group="A"

Sample size	356
Correlation coefficient r	-0.1061
Significance level	P=0.0454
95% Confidence interval for r	-0.2078 to -0.002187

Scatter diagram



Correlation FIG 11.

Variable Y	PDW
Variable X	HbA1c
Filter	Group="A"

Sample size	356
Correlation coefficient r	0.05144
Significance level	P=0.3332
95% Confidence interval for r	-0.05279 to 0.1546

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Scatter diagram