Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2016; 4(3D):877-886 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2016.v04i03.050

Original Research Article

Platelet Indices and Endothelial Dysfunction in Patients of Diabetes Mellitus Type 2

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Abstract: Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Macro- and microvascular disease are currently the principal causes of morbidity and mortality in patients with type 1 and type 2 DM. DM has been recognized as a 'prothrombotic tendency' with increased platelet reactivity. This enhanced reactivity has been postulated to play a role in the microvascular complications of diabetes. The study was aimed at analysis of platelet indices -MPV and PDW and its relation with endothelial dysfunction in patients of DM. This study included 100 subjects, 50 diabetics and 50 healthy subjects from the hospital as controls. Anticoagulated blood was collected and analyzed in an automated blood cell counter for platelet count, Mean platelet volume (MPV) and platelet distribution width (PDW). Endothelial dysfunction was assessed indirectly by vessel reactivity using non-invasive automated digital thermal biofeedback mechanism. In results we studied 50 healthy (mean age 56.56±11.06 years) and 50 diabetics (mean age 56.8±7.96). MPV 8.96±1.93 fL was significantly higher and PDW 19.11±1.45 10(GSD) was raised in diabetic patients compared to the controls (MPV 7.57±2.54 fL and PDW 18.93±1.07 10(GSD). Post cuff deflation temperature rebound (TR) was significantly lower in patients with DM type 2 as compared to normal subjects indirectly reflecting endothelial dysfunction. The diabetic subjects were unable to achieve the basal temperature even after 2 minutes of cuff deflation as compared to controls where temperature rebound was achieved within 1 minute. This difference was statistically significant (P = <0.005). In conclusion the Platelet indices; MPV was significantly raised where as PDW was higher in diabetics and this was directly related to delay in temperature rebound reflecting endothelial dysfunction.
Keywords: Diabetes mellitus, MPV, PDW.

INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Depending upon the etiology of DM, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with DM and on the health care system [1].

DM has been recognized as a 'prothrombotic tendency' with increased platelet reactivity [3]. This enhanced reactivity has been postulated to play a role in the microvascular complications of diabetes. MPV reflects either, changes in platelet stimulation or the rate of platelet production. PDW is a measure of platelet heterogeneity, which in turn may be due to platelet aging or heterogeneous demarcation of megakaryocytes [3].

The common feature underlying the diverse complications of diabetes is vasculopathy both "micro" and "macro", characterized by progressive narrowing of lumen as well as abnormal permeability to proteins [4]. Diabetes and its complications have been used as a model for accelerated aging. The ongoing biochemical process of non-enzymatic cross linking of several types of macromolecules, including proteins and nucleic acids, leads to modification and then decline in structure and function of these molecules, as the cross links accumulate both extracellularly and intracellularly over the time. The prime example would be cross linkage of collagen, which is thought to lead to typical phenomena observed in aging, such as increased susceptibility to atherosclerosis, osteoporosis, decreased

joint elasticity, the formation of cataracts, and cardiac enlargement [5, 6].

Macro- and microvascular disease are currently the principal causes of morbidity and mortality in patients with type I and type 2 DM. Loss of the modulatory role of the endothelium may be a critical and initiating factor in the development of diabetic vascular disease [2].

Various non-invasive methods are available to assess the effect on endothelial physiology, such as Doppler technique and use of nitric oxide [7]. Thermal biofeedback is a useful adjunctive technique for enhancing cutaneous blood flow (Vascular reactivity) in patients with vascular complications of diabetes. Hand temperature change has been monitored in thermal biofeedback sessions in earlier studies and has found to have significant changes. The advantage of using biofeedback mechanism are, its easy to use, it can read changes of 1/10 of a degree of Fahrenheit, Also it can read changes in very short time interval as short as milliseconds [7].

Platelets play an important role in the integrity of normal homeostasis, and MPV is the indicator for its function. The large platelets contain more dense granules, are more potent than the smaller platelets, and are hence more thrombogenic. Both the size and number of granules in platelets in circulation are under independent hormonal control and do not change during the life span of the platelet. Increase in MPV has been documented in patients with metabolic syndrome, stroke and DM.MPV, a determinant of platelet function, is a newly emerging risk factor for atherothrombosis [3, 8].

The present study was aimed at evaluating the platelet indices (MPV, PDW) and digital thermal biofeedback response for endothelial dysfunction in patients with diabetes mellitus type 2 and comparing them with healthy controls. Also and attempt was be made to assess the difference in the platelet indices between diabetics with and without micro-angiopathy. Statistical analysis was performed to evaluate the utility of above features being indicators of earliest onset of diabetic microvascular complications.

MATERIALS AND METHODS:

The study was conducted in the Department of Medicine, Himalayan Institute of Medical Sciences (HIMS), Swami Ram Nagar, Dehradun, over a period of 12 months. Subjects were recruited from OPD and IPD of the department of medicine, after obtaining informed written consent and approval from the ethical committee of the institution

Study Design: Observational, Descriptive, Cross sectional study.

Sample Size: 50 diabetics and 50 healthy subjects

Selection of Subjects:

Inclusion Criteria - Patients of Diabetes mellitus type 2 as per American diabetic association criteria.

Exclusion Criteria - Patients with abnormal platelet number and / or abnormal hematocrit.

Detailed clinical history, with special emphasis on symptoms related to complications of DM, was elicited in the study group. A thorough clinical examination and relevant clinical investigations were conducted for detection of diabetic complications in the study group. Following routine investigations were done - Complete Haemogram including Hb, TLC, DLC, Platelet count, MPV, PDW, analyzed by the ABOTT CELLDYNE 3700 machine in the pathology lab of Himalayan hospital. (Which is maintained under a strict quality check for the instrument as directed by the laboratory protocol of the institute, 2 level controls in a day for accuracy and a sample is run 10 times a day for precision study.) FBS/RBS, HbA1c, Lipid profile, S.Creatinine, Urine albumin, ECG and detailed fundus examination. Thermal biofeedback technique to measure vascular reactivity in upper extremity was done by the machine physio-PAC 8-channel polygraph manufactured by MEDICAID. The technique begins with the blood pressure recording (Welch Allyn Tycos®509 Mobile Aneroid Sphygmomanometer) in right / left upper limb after resting in sitting position for 10 minutes in ambient temperature of 22-24 C. Digital temperature recording (DTR) was performed during 2 minutes of stabilization. Then suprasystolic cuff occlusion of the same arm is done for 3-5 minutes to create relative ischemia by inflating the cuff, 40mmHg greater than systolic blood pressure. During the cuff occlusion fingertip temperature falls because of the absence of warm circulating blood in the ischemic area. Then the BP cuff is released causing rush of blood into fore arm and hand, leading to temperature rebound which is directly proportional to the reactive hyperemia response, continuous temperature recording was done by the finger probe throughout the process and continued till 6 minutes after cuff deflation. This data was automatically digitalized by the machine (physio-PAC 8-channel polygraph manufactured by MEDICAID). The faster the temperature rebound (TR) higher the vascular reactivity. The difference between the temperature and time taken for temperature to come back to baseline was recorded on a graph for calculation [9]. Patients with evidence of retinopathy/Neuropathy/Nephropathy were considered as patients having microangiopathy. Similar numbers of

healthy subjects were also subjected to thermal biofeedback technique, which served as a control

Data Management and Statistical Analysis:

Interpretation and analysis of observed results was carried out by using SPSS software (Statistical Package for Social Sciences) version 17 and Microsoft excel. Non-parametric test like chi square test was applied to see the association between the variables.

RESULTS:

Patient characteristics

The mean age of diabetic patients was 56.8±7.96 years compared to 56.56±11.06 years in control subjects. There was no statistical difference in age between diabetic subjects and controls. (P value = 0.901). There was an incidental male predominance in both groups with 37 males in the study group and 34 males in the control group where as there were 13 females in the study group and 16 females in the control group (Table 1). All the diabetics except four had either one or more micro/macrovascular complications.

Table 1: Patients characteristics

	CASE (n=50)	Control (n=50)	P value
AGE (Years)	56.8±7.96	56.56±11.06	0.901
MALE	37 (74%)	34 (68%)	0.785
FEMALE	13 (26%)	16 (32%)	0.623
SBP	128.32±14.13	129.6±2.82	0.531
DBP	77.4±9.64	81±4.62	0.019
BMI (Kg/m2)	26.16±5.24	23.69±1.76	0.002
MPV	8.96±1.93	7.57±2.54	0.002
PDW (10 GSD)	19.11±1.45	18.93±1.07	0.48

BMI- body mass index, SBP-systolic blood pressure, DBP-systolic blood pressure, MPV- mean platelet volume, PDW-platelet distribution width

Table 2: MPV in diabetics and controls according to their BMI

BMI (kg/m2)	Case		Control		P value
	No.	MPV(fL)	No.	MPV(fL)	
Group 1: 18.5 – 25 (healthy)	26	8.98±2.12	40	8.27±2.21	0.199
Group 2: 25.1 – 30 (overweight)	20	8.60±1.68	10	8.38±1.60	0.733
Group 3: > 30 (obese class1)	4	8.33±1.96	0	0	Nil

The BMI of diabetics was observed to be higher than that of the control group.

Table 3: PDW in diabetics and controls according to their BMI

BMI (kg/m2)	Case		Control		P value
	No.	PDW (10 GSD)	No.	PDW (10 GSD)	
18.5 – 25	26	18.16±1.51	40	18.12±1.25	0.907
25.1 – 30	20	18.10±1.35	10	18.01±1.10	0.856
> 30	4	18.15±1.97	0	0	nil

Table 4: Platelet indices MPV and PDW in diabetic hypertensives and non hyper tensives.

	MPV (fL)	PDW (10 GSD)
Hypertensive (n=16)	8.57±1.05	18.94±1.31
Normotensive (n=34)	8.16±2.25	18.25±1.52
P value	0.245	0.016

Table 5: Platelet indices mean platelet volume (MPV) and platelet distribution width (PDW) in smokers and non smoker diabetics

	MPV(fL)	PDW (10 GSD)
Smoker (n=8)	7.87±1.30	18.73±1.29
Non smoker (n=42)	7.93±1.99	17.96±1.44
P value	0.935	0.166

Table 6: Platelet indices MPV and PDW in alcoholic and non alcoholic diabetics

	Alcoholic (n=10)	Non alcoholic (n=40)	P value
MPV (fL)	7.79±1.69	8.0±2.0	0.761
PDW (10 GSD)	18.87±1.41	17.93±1.42	0.066

Table 7: Comparison of diabetics and controls with respect to glycemic control (HBA1c) and platelet indices MPV and PDW

	Case (n=50)	Control(n=50)	P value
HbA1c (%)	9.70±2.74	5.62±0.42	0.001
PDW (10 GSD)	18.09±1.47	18.6±1.76	0.119
MPV (fL)	8.96±1.93	7.57±1.54	0.001

Platelet indices

The platelet indices MPV and PDW were compared between the diabetic patients and the control subjects as tabulated in table 8.

Table 8: Platelet indices mean platelet volume (MPV) and platelet distribution width (PDW) in diabetic and control group.

	Case (n=50)	Control (n=50)	P value
MPV(fL)	8.96±1.93	7.57±2.54	0.002
PDW 10(GSD)	19.11±1.45	18.93±1.07	0.48

MPV-Mean platelet volume, PDW-platelet distribution width

On statistical analysis MPV was significantly higher and PDW was raised in diabetic patients in comparison to the control subjects. There was statistically significant difference in the MPV even though the same was not seen for PDW. Platelet indices were also compared according to the glycemic control of the diabetic patients as shown in table 3. The platelet indices MPV and PDW were observed to be higher in patients with poor glycemic control.

Table 9: Glycemic control and platelet indices MPV and PDW in diabetics

HbA1c (%)	MPV (fL)	PDW (10 GSD)
5.97 – 6.80 (n=0)	-	-
6.81 – 7.63 (n=9)	7.34±0.64	18±1.45
7.64 & above (n=41)	8.02±2.06	18.7±1.43
P value	0.288	0.159

Table 10: Duration of diabetes and Platelet indices MPV and PDW

Duration of diabetes	Newly diagnosed (n=7)	Known diabetes (n=43)	P value
MPV (fL)	7.08±2.20	7.94±1.91	0.863
PDW (10 GSD)	17.34±1.04	17.91±1.42	0.017

Platelet indices MPV and PDW were also compared according to the duration of diabetes as shown in table 10. Even though both MPV and PDW were seen to be higher with increase in duration of diabetes but there was no statistical significance seen between them.

Prediction of diabetic vascular complications

Table 11: Thermal biofeedback response at basal temperature in diabetics in both sexes

Sex	Basal temp		Temp after 2 min		P value
Male	37	88.04±1.43	37	84.88±3.12	0.001
Female	13	87.90±3.45	13	86.15±3.04	0.002

Both the study group as well as the controls were made to undergo a thermal biofeedback response test, where it was observed that the diabetic patients could not achieve their basal temperature even after 2 minutes from cuff deflation where as in the control group the subjects could achieve the basal temperature by the end of 60 seconds of cuff deflation. The mean basal fingertip temperature of males (n = 37) was observed to be 88.04±1.43 F and the mean temperature at the end of 2 minutes from the time of deflation of the cuff was 84.88±3.12 F. The mean basal temperature in diabetic females (n = 13) was 87.90±3.45 F and the mean temperature after a 2 minutes from the time of deflation of cuff was observed as 86.15±3.04 F. Hence both male and female diabetics could not achieve their basal temperature at the end of 2 minutes of cuff deflation. This was seen to be statistically significant with P = 0.001 and 0.002 for males and females respectively. (Table 11)

Table 12: Thermal biofeedback response - control group in both sexes

Sex	Basal temperature (f)		Control Temperature after 2 minutes (f)		P value
Male	34	84.88±3.12	34	84.71±2.71	0.811
Female	16	83.05±2.49	16	83.23±2.40	0.836

In the normal subjects there was no difference in the TR suggesting normal endothelial function.

Table 13: Platelet indices MPV, PDW, and thermal rebound in diabetics with micro and macroangiopathy.

	Microangiopathy (n=31)	Macroangiopathy (n=15)	P value
MPV (fL)	7.63±1.50	7.79±1.78	0.751
PDW (10 GSD)	17.97±1.42	17.96±1.48	0.982
Basal temperature (F)	88.60±2.21	87.30±3.26	0.117
Temprature at end of 2 minutes after cuff deflation (F)	87.52±2.87	87.0±2.19	0.539

Platelet indices MPV and PDW were compared in diabetic patients with mico and macroangiopathy. The data obtained from the thermal biofeedback response was also compared for diabetics with micro and macro angiopathy. There was no significant statistical difference seen. (Table 13)

Table 14: Platelet indices - MPV and PDW in diabetics who achieved basal temperature within 2 minutes and more than 2 minutes

	Basal temperature achieved at 2 min (f) (n=11)	Basal temperature not achieved at 2 min (f) (N=39)	P value
MPV (fL)	8.30±1.22	8.86±1.86	0.352
PDW (10 GSD)	17.29±1.21	18.16±1.52	0.087

On inter group comparison of the TBFBR (thermal biofeedback response) and platelet indices it was observed that diabetics who did not achieve their basal temperature within 2 minutes from the period of deflation of the cuff had higher platelet indices MPV and PDW than those who did. (Table 14)

The diabetic patients were observed to have higher platelet indices MPV and PDW as compared to the control group. Also due to increased endothelial dysfunction or decreased vascular reactivity the diabetic patients could not achieve their basal fingertip temperature within a period of 2 minutes from the time of cuff deflation.

DISCUSSION:

DM is a 'prothrombotic state' associated with accelerated atherosclerosis and inflammation. Patients with diabetes, particularly those with type 2 DM, have been shown to exhibit increasing platelet reactivity. This has been attributed to both insulin resistance and insulin deficiency. Insulin has been shown to antagonize the effect of platelet agonists like collagen, adenosine diphosphate, epinephrine and platelet activating factor [10]. Hyperglycemia con- tributes to heightened platelet reactivity directly as well as through glycation of platelet proteins. In addition, hypertriglyceridemia also increases platelet reactivity [11]. Enhanced platelet aggregation has been implicated in the development of micro- and macro- vascular disease in patients with DM. [2]. An earlier study has shown higher MPV values in diabetic patients with retinopathy and other vascular complications. Microvascular complications include effects on small vessels, arterioles, and capillary venules. Complications starts early in the pathogenesis of DM type 2 and accounts for morbidity, in the form of retinopathy, neuropathy and nephropathy [5].

There have been a few studies in the available literature on platelet indices in patients with DM. An increase in MPV has been documented in patients with metabolic syndrome, stroke and DM [12]. Increased MPV has also been shown to be an independent risk factor for myocardial infarction, cerebral ischemia and transient ischemic attack, platelet indices; Mean platelet volume (MPV) reflects either changes in platelet stimulation or the rate of platelet production. Platelet distribution width (PDW) is a measure of platelet heterogeneity, which in turn may be due to platelet aging or heterogeneous demarcation of megakaryocytes [13]. In earlier studies, MPV was found to be significantly higher in diabetic patients and it was hypothesized that platelets with altered morphology are likely to be associated with increased risk of vascular complications in diabetes [14]. Larger platelets contain more dense granules and hence are more potent and thrombogenic [15].

Platelet activation is known to occur in patients with concomitant cardiovascular disease, Hypertension [16] and diabetes [17] most likely due to platelet activation secondary to vascular injury. Additionally abnormal glycation is thought to contribute to abnormalities of platelet function in diabetic patients [15, 16, 17].

Discriminant analysis was performed taking presence and absence of vascular complications in subjects in a study with DM as an outcome variable and age of the patient, duration of DM, Platelet count, MPV, PDW was taken as input variables. At the last step of analysis, only two variables were present in the model obtained. Of the group of diabetics with microvascular complications, 78.6% were correctly classified by this method [3].

The majority of studies examining "endothelial function" in vivo in the last decade have involved the measurement of endothelium-dependent dilatation, in large part because techniques have been developed to assess this particular aspect of endothelial physiology. The endothelium, however, has a multiplicity of functions beyond the regulation of vessel tone, as noted above. Thus, articles reporting on "endothelial function" and "dysfunction" on the basis of the measurement of endothelium-dependent dilatation give insights to only one aspect of endothelial physiology, albeit an important one. Little work has been done to date on how the various endothelial functions correlate with each other in disease states (for example, how impaired endothelium-dependent dilatation, regulation of endothelial cell adhesion molecule expression, and release of key hemostatic regulatory molecules relate to one another [2, 18].

In previous studies non-invasive techniques used to assess endothelial dysfunction vascular reactivity were used (vasodilative reactivity) for analysis of fingertip temperatures. This served as a surrogate marker of blood flow changes that result from vascular reactivity. Vascular reactivity is a vital component of vascular function that enables the circulatory system to respond to physiologic and pharmacologic stimuli that require adjustments of blood flow and alterations of vessel tone and diameter. Vascular reactivity occurs in two forms – vasoconstrictive and vasodilative – and can be exhibited at both the macrovascular and microvascular levels [5, 19].

In many countries including the Indian sub continent there is a growing public concern due to the escalation of number of people with diabetes while complication rates and associated diseases amongst diabetics are high. In addition there is high prevalence of

complications such as blindness, end stage renal disease, lower extremity amputations as well as premature cardiovascular disease, stroke and premature mortality related to poor control of blood glucose [20]. In DM there is a disturbance of intermediary metabolism manifesting as chronic sustained hyperglycemia primarily due to either an absolute or a relative lack of insulin. This may be accompanied by other biochemical disturbances and the presence of progressive diabetic tissue damage with micro vascular and macro vascular complications. The world today is witnessing an epidemic of diabetes globally and nationally. Diabetes Mellitus with its complications has become the most important contemporary and challenging health problem. The prevalence in India is currently reported to be around 13 – 15 % and by 2025, it is estimated that approximately 55 million Indians will be diabetic [21].

The present study was done on 50 diabetics and 50 healthy age and sex-matched controls were randomly selected to find out platelet indices - MPV, PDW and presence of endothelial dysfunction. In the diabetics there were 37(74%) males and 13(26%) female. In the control group there were 34(68%) males and 16(32%) females. There was no statistical difference in age between diabetics and controls (P = 0.901).

The BMI for diabetics and controls showed a statistical significance with diabetics having a higher mean BMI 26.16±5.24 kg/m2 than that of the control 23.69±1.76 kg/m2 (P value=0.002). Risk of non-insulin-dependent diabetes mellitus has been strongly associated with obesity as pointed out in a study carried out by June. M. Chal *et al.;* where they investigated the relation between obesity, fat distribution, and weight gain through adulthood and the risk of diabetes mellitus. They found a strong positive association between overall obesity as measured by body mass index and risk of diabetes. There was a significant difference between the mean BMI of diabetics and controls with diabetics having a higher mean [21].

As shown in table 2 there were increased micro and macrovascular complications with progression of duration of diabetes. This was also reported by the study conducted by Jindal *et al.;* [22]. Also similar results were reported by a study done by Viswanathan Mohan *et al.;* [23]. Where they found high prevalence of complications related to Type 2 diabetes in the patients with long standing diabetes.

Mean platelet volume (MPV) is an index of platelet activation and PDW is an indication of variation in platelet size, which is a sign of active platelet release. In our study statistical significance (P value <0.05) was observed in the platelet indices MPV in diabetics with mean MPV 8.96±1.93 fL and the mean MPV in controls which was 7.57±2.54 fL. Also, the PDW was reported to be higher in diabetics with mean PDW 19.11±1.45 (10 GSD) and the mean PDW in controls was 18.93±1.07 (10 GSD) but it was not statistically significant. Similar results were reported by Sonali Jindal *et al.;* [2] where they reported the platelet indices to be higher in diabetics (n=75), mean PDW 15.349±3.14 as compared to that of the controls (n=50), mean PDW 17.25±3.56. Similar results were reported by Zeliha Hekimsoy *et al.;* [24] in a study titled "Mean platelet volume in Type 2 diabetics" in which MPV was significantly higher in diabetics compared to age- and sex-matched non diabetic healthy controls [10.62 ± 1.71 fL vs. 9.15 ± 0.86 fL (P= .001)], respectively.

Macro- and microvascular disease are currently the principal causes of morbidity and mortality in patients with diabetes mellitus. Loss of modulatory role of the endothelium may be critical and initiating factor in the development of diabetic vascular disease. Endothelial cells actively regulate basal vascular tone and vascular reactivity in physiological and pathological conditions, by responding to mechanical forces and neuro-humoral mediators with the release of a variety relaxing and contracting factors [25]. We studied the endothelial dysfunction (vascular reactivity) by way of non-invasive thermal biofeedback mechanism. In temperature biofeedback (TBFB), skin temperature is monitored by means of a thermistor fastened on the fingertip pad or other relevant site. Because cutaneous temperature is closely linked to capillary flow, successful TBFB alters blood flow.

We observed that the mean MPV in smokers (n=8) with diabetes was 7.87±1.30 fL as compared to the mean MPV in non-smoker (n=42) with diabetes was 7.93±1.99 fL. (P value=0.935) The mean PDW in smoker diabetics was 18.73±1.29 (10GSD) and the mean PDW for non-smoker diabetics 17.96±1.44 (10GSD) (P value=0.166). Similar results were seen in a study done by Arslan. E *et al.;* [26] where they studied Smoking and cholesterol levels are two significant causes of atherosclerosis. Only limited numbers of studies showing the effect of smoking on platelet function are published. However, no significant difference was found for MPV between the groups (smoking: 8.57+/-0.8 fl; nonsmoking: 8.67+/-0.8 fl; p=0.66).

In our study the MPV and PDW in diabetic patients who consumed alcohol (n=10) and non-alcoholics (n=40), mean MPV in alcoholic diabetics was 7.79±1.69 fL and the mean MPV of non-alcoholic diabetics was 8.0±2.0 fL (P value= 0.761). The mean PDW in alcoholic diabetics was 18.87±1.41 (10 GSD) and in non-alcoholics the mean PDW was 17.93±1.42 (10 GSD), (P value = 0.066). This was similar to a

study conducted by Luzzatto.G [26], which was titled "Increased proportion of giant platelets and platelet distribution width are better indicators of altered platelet homeostasis than mean platelet volume in liver cirrhosis". It was concluded that PDW were markedly increased in alcoholics with liver cirrhosis as compared to controls, while MPV was not significantly different. Not many studied have been done to evaluate platelet indices MPV and PDW in alcoholics. In our study even though there was significant difference in platelet indices of alcoholics and non-alcoholic diabetics, this was not statistically significant (P > 0.05).

Thermal biofeedback response was also observed in diabetic and non-diabetic alcoholics. The mean basal temperature in alcoholic subjects was 88.58±1.76 f, Mean basal temperature in non alcoholics was 88.48±2.99 f (P value =0.920). The mean temperature at the end of 2 minutes from cuff inflation in alcoholic diabetics was 87.28±1.96 f and mean for non-alcoholics was 87.66±3.39 f (P value=0.736). There has been no study done to test thermal biofeedback response in diabetes keeping alcohol as a factor. The average delay in achieving the basal temperature after 2 minutes from the time of cuff deflation was 4-5 minutes. There were some diabetics in both subgroups that were unable to achieve their basal temperature even after a period of 6 minutes.

Endothelial dysfunction and damage can be detected by various methods: a measure of its ability to respond appropriately to simulated increased shear force (i.e. flow mediated dilatation) or the concentrations of various molecules that it produces may each give an indication or abnormality There have been very few studies specifically designed to investigate the impact of various types of neuropathies on thermal biofeedback mediated response. One of the purposes of this study was to investigate the parameters of thermal biofeedback response to look for endothelial dysfunction. Healthy population achieved the basal temperature within one minute after deflation of the B.P (blood pressure) cuff but we found that diabetic patients took a longer duration than healthy adults to achieve the basal temperature at the end of 2 minutes. Similar results were reported by an earlier study by K Bhargava *et al.;* [9] where they studied endothelium dependent brachial artery flow mediated vasodilatation in diabetics. They reported impaired flow mediated dilatation in diabetics as compared to controls (P value = 0.001) and the degree of was directly related to duration of diabetes, which is also congruent with our study. 10(20%) diabetics (n=50) could not achieve the basal temperature upto a period of 5 minutes and 40(80%) achieved the basal temperature after a delayed average time of 4-5 minutes. Whereas all the healthy subjects (n=50) could achieve the basal temperature in less than 3 minutes from the time of B.P (blood pressure) cuff deflation.

Endothelial dysfunction was studied in patients with hypertension by Thermal biofeedback response and the mean basal temperature was 88.51±2.38 F and the mean temperature after 2 minutes of cuff deflation was 87.44±2.45 F. This points out that they hypertensive diabetic patients could not achieve the basal temperature in view of endothelial dysfunction (impaired vascular reactivity). Similar results were also seen in a study conducted by Jiang.Li [26] in a study titled Non invasive detection of endothelial dysfunction in patients with essential hypertension. They also used the same reactive hyperemia technique produced by cuff inflation and deflation and measured brachial artery dilatation. The mean dilatation in controls was 12.4 ± 2.9 mm for controls and 4.6 ± 2.8 mm in hypertensives (P value = 0.001).

We observed that MPV in diabetics was significantly higher in diabetics (n=50) with mean MPV 8.96±1.93 fL than in controls with mean MPV 7.57±2.54 fL (P value = 0.002). This is similar to the result in many studies such as one carried out by Zuberi B *et al.;* [27], They conducted a study to compare MPV in patients with diabetes mellitus and non-diabetic subjects and the difference of MPV among the non DM and DM groups was highly significant (P value = 0.000 and confidence interval of -0.67).

The platelet indices PDW (Platelet distribution width) was also higher in diabetic patients with diabetics having a mean PDW of 19.11±1.45 (10 GSD) and the mean PDW of 18.93±1.07 (10 GSD) in the control group (P value=0.119) was which is similar to the study done by Sonali Jindal *et al.*[2]*;* where there mean PDW in diabetics was 17.25 ± 3.56 and the mean PDW in their control group was 15.34±3.14 (P value = 0.002).

CONCLUSION:

Our study highlights the differences in platelet indices MPV and PDW in diabetics and non-diabetics along with the presence of endothelial dysfunction (impaired vascular reactivity) in diabetics. Platelet indices (MPV,PDW) when co-related with thermal biofeedback in diabetics showed significantly higher values indicating a positive co-relation between higher platelet indices and endothelial dysfunction. DM is the one of the most common chronic diseases across the world and the number of diabetics is on the rise and this causes a tremendous burden on the healthcare infrastructure. Endothelial dysfunction is a key early event in the atherogenesis and is known to appear long before the formation of structural atherosclerotic changes [28]. Assessment of endothelial dysfunction by observing the MPV and PDW which otherwise are not

given as much significance when a complete hemogram is perceived and provide valuable insight into pre intrusive phase of endothelial dysfunction in diabetic individuals hence enabling us to provide early available prevention to endothelial dysfunction and atherosclerotic disease in diabetes.

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