PLATELET INDICES AMONG SUBJECTS WITH AND WITHOUT DIABETES MELLITUS AND HYPERTENSION: A CROSS-SECTIONAL ANALYSIS AT KARACHI, PAKISTAN

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ABSTRACT

Objective: To measure differences of platelet indices in subjects with and without diabetes mellitus and hypertension

Methodology: This was a cross-sectional analysis from Jan-11 to Feb-2012. From a target population of non-pregnant adult subjects being advised a fasting blood glucose or OGTT for confirming or excluding diabetes, we finally selected 820 individuals for inclusion into study. Fifty known diabetics were also included in the study. Subjects were formally interviewed and sampled for fasting blood glucose, and platelet indices (including platelet count, plateCrit (PCT), mean platelet volume (MPV) and platelet distribution (PDW). The results of platelet indices were compared between 4 groups based on OGTT. Platelet indices were also compared between groups based upon post-load glycemic status and hypertension.

Results: Age and MPV showed slight positive and significant correlations with fasting blood. [Age: r2=0.117 (p<0.001) and MPV: r2=0.116 (P=0.001); other platelet indices did not show significant correlations. Out of the 4 platelet indices studied, MPV and PCT were found to significantly increase from normoglycemia to individuals with established diabetes mellitus in one way ANOVA analysis. Subjects demonstrating post load hyperglycemia (n=47) had higher mean platelet volumes than individuals having post load normoglycemia (n=30) [MPV: 9.63 + 1.51vs 8.90 + 0.98, p=0.012]. Hypertensive subjects did not demonstrate higher MPV results than normotensive subjects in our study.

Conclusion: Mean platelet volume and platecrit increases across various grades of hyperglycemia. However, the changes become quite prominent in subjects having established diabetes with marked hyperglycemia. Post load hyperglycemia was more predictive of rises in mean platelet volumes.

Keywords: Platelet count, platecrit (PCT), mean platelet volume (MPV), platelet distribution width (PDW), fasting plasma glucose, diabetes mellitus, hypertension.

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INTRODUCTION

Cardiovascular diseases constitute a major source of mortality and morbidity along with massive effects on national and international economies[1]. Diabetes and hypertension remains as the most imminent disease contributing to the cardiovascular disease burden due to ongoing low grade inflammation[2,3]. While the scientific search expands to introduce and incorporate newer diagnostic and therapeutic targets to address these disease processes, it remains vital to identify the key pathological culprits in the disease pathogenesis[4].

Traditionally the measurement of blood glucose and other markers of glycemia provide some insight about the disease presence, but still the end stage or the underlying direct mechanics involving the accelerated process of atherosclerosis are needed to be explored[5]. The usual final outcome of an atherosclerotic process, causing significant suffering to human community is the process of enhanced thrombosis with ultimate plaque rupture[6]. Platelets, their receptors and vessel wall dynamics become one of the leading players in defining the sequences to the above process[7]. However, before such a process shapes up, there is evidence to state that some morphological and biochemical changes do appear around vessels and platelets[8].

Major function of platelets includes the physiological part of thrombotic process, while the pathological plaque formation occurs once these platelets develop enhanced stickiness to the vessel walls[9]. To possess the quality of enhanced stickiness, they must have gone through some morphological changes[8]. Recently, literature has shown the utility of some of the platelet indices including their mean volumes in specific, to be significantly modified due to underlying atherogenic diseases of metabolism[10]. However, the association with other markers like platelet distribution width (PDW) has not been much explored. Secondly, once antiplatelet drugs are being recommended to the patients for prevention of an ischemic event, then it is also felt that there must be some platelet structural changes which prevent the development of such metabolic processes[11]. Thirdly, the exact relationship between various platelet indices, including platelet itself and glycemic status also require elaboration in order to optimize the utilization of such indices in clinical use because some studies do advocate minimum yield for routine use[12]. Finally there is a strong possibility that ethnicity and race may reveal differing data about the platelet indices and their sort associations with diabetes and hypertension[13]. In this connection, the search of local data suggest only one study by Zuberi et al, utilizing one of the platelet indices for measuring the differences at various levels of blood glucose in our local population[14].

With this background in mind a study was planned to measure the platelet count and indices among subjects with and without diabetes mellitus and hypertension.

METHODOLOGY

This cross-sectional analysis was conducted from Jan-2011 to Feb-2012 at Karachi, Pakistan. The target population was subjects presenting at the department of pathology for evaluation of their fasting blood glucose as an alone advised test or as part of OGTT. Based upon non-probability convenience sampling a final total of 820 subjects were enrolled in the study after brief explanation of study purpose and formal signing of consent for inclusion into the study. Subjects who were pregnant, having some acute infectious disease, not observing proper medical fasting, on current anti-platelet therapy and indoor cases were excluded from the study. Initially we only enrolled un-diagnosed diabetics but later to compare our results fifty diagnosed type-2 diabetics were also included. The selected subjects were briefly interviewed for clinical details especially for presence of hypertension and ischemic heart diseases.

Up to 4 ml of blood was collected in heparinized tube for estimation of fasting blood glucose, and 3 ml in EDTA container for measurement of platelet indices (including platelet count, platecrit (PCT), mean platelet volume (MPV) and platelet distribution (PDW).

Oral glucose tolerance test-Subjects (n=77) who were indicated OGTT from clinics were also inclusive in the above sample. They underwent OGTT with 75 grams of glucose as per the standard protocol.

Glucose was analyzed by hexokinase method on a random access clinical chemistry analyzer (Hitachi-902). Platelet indices were measured by an 18-part hematology analyzer.

During analysis samples (n=50) having visible hemolysis, chylous or having visible clots were excluded from the study.

a. Glycemia status- The results of fasting blood glucose were categorized into following groups: Group-1(Normal glucose tolerance: Fasting blood glucose results < 5.6 mmol/L), Group-2 (Impaired fasting glucose: results between 5.6 to 6.9 mmol/L), Group-3 (Newly diagnosed diabetes mellitus: results > 6.9 mmol/L), and Group-4 (Diagnosed type-2 diabetes mellitus)

b. 02 hr OGTT results-

Group-1: 02 hour OGTT result (< 7.8 mmol/L) [Normal glucose tolerance (NGT)]

Group-2: 02 hour OGTT result (> 7.8 mmol/L) [Post load hyperglycemia], including both subjects with impaired glucose tolerance [IGT] and diabetes mellitus.

All data were entered into Excel program (Microsoft office-2007) and SPSS Version-15. Descriptive statistics in terms of mean and SD/95% confidence intervals were calculated for age. Frequencies were calculated for gender. Correlation of platelet indices with measures of glycemia and platelet indices were calculated by Pearson's correlation. Comparison of differences between various platelet indices between groups based upon fasting glycemia, hypertension and 2 hour post load glycemia were measured by one way ANOVA and independent sample test respectively. Later the differences in platelet indices were evaluated between groups based upon fasting hyperglycemia by keeping gender and age fixed by univariate GLM (General linear model).

RESULTS

Mean age among our data set was 43.41 $\pm$11.51 years. 52.7 % were male while 47.3 % were female. Gender based differences for age, fasting blood glucose, platelet count, mean platelet volume (MPV), platecrit (PCT), and platelet distribution width (PDW) are shown in table-1. Out of the parameters evaluated, only age and MPV showed slight positive and significant correlations with fasting blood glucose. [Table- 2]

Mean platelet volume (MPV) and platecrit (PCT) were found to rise from normoglycemia to individuals with established diabetes mellitus as shown in table-3. The post hoc-comparisons showed this rise reaching sta-

tistically significant once the transition occurred from newly diagnosed diabetics (group-3) to subjects with established diabetes (group-4). However, once gender is defined as a random variable in the GLM model the differences in MPV become statistically significant across various levels of glycemia especially among the male subjects. [Figure-1] Subjects demonstrating post load hyperglycemia (n=47) had higher mean platelet volumes than individuals having post load normoglycemia (n=30) [MPV: 9.63 ± 1.51vs 8.90 ± 0.98, p=0.012] as shown in figure-2. Hypertensive subjects did not demonstrate higher MPV results than normotensive subjects in our study; however, once the diagnosis of hypertension augments diabetes mellitus, MPV further increases. [Figure-3]

DISCUSSION

Our study has shown that mean platelet volumes (MPV) and the percentage volume of platelets i.e.,

Table 1: Gender differences among various outcome measures (n=870)

Parameter	Gender	Mean	Std. Deviation	Sig
Age in years	Male	45.43	11.75	<0.001
	Female	41.17	10.83	
Fasting blood glucose (mmol/L)	Male	6.22	2.33	0.132
	Female	6.01	1.80	
Platelet count	Male	237	66.63	<0.001
	Female	287	77.99	
Platecrit (PCT)	Male	0.211	0.07	<0.001
	Female	0.2580	0.09	
Mean platelet volume (MPV)	Male	9.62	1.35	0.025
	Female	9.42	1.28	
Platelet distribution width (PDW)	Male	14.02	0.71	< 0.001
	Female	13.84	0.73	

Table 2: Bivariate Pearson's correlations between fasting blood glucose and different platelet parameters (n=870)

S.No	Parameter	Correlation co-efficient (r2)	Significance
1.	Age in years	0.117**	0.000
2.	Platelet count	-0.042	0.219
3.	Platecrit (PCT)	0.008	0.808
4.	Mean platelet volume (MPV)	0.116**	0.001
5.	Platelet distribution width (PDW)	0.021	0.543

**Correlation is significant at the 0.01 level (2-tailed)

Table 3: Differences in platelet indices among groups formulated according to patients' fasting glycemic status (n=870)

S.NO	Parameter	Group	N	Mean	95% Confidence interval		Sig. (One way ANOVA)
					Upper bound	Lower bound	
1.	Platelet count	Normal glucose tolerance (FBG < 5.6 mmol/L)	360	257	249	265	0.320
		Impaired fasting glucose (FBG between 5.6 & 6.9 mmol/L)	369	266	257	274	
		Newly diagnosed diabetes mellitus (FBG > 6.9 mmol/L)	91	252	238	265	
		Diagnosed diabetes mellitus on treatment	50	259	237	281	
2.	Platecrit (PCT)	Normal glucose tolerance (FBG < 5.6 mmol/L)	360	0.22	0.22	0.23	0.015
		Impaired fasting glucose (FBG between 5.6 & 6.9 mmol/L)	369	0.24	0.23	0.25	
		Newly diagnosed diabetes mellitus (FBG > 6.9 mmol/L)	91	0.23	0.21	0.25	
		Diagnosed diabetes mellitus on treatment	50	0.25	0.23	0.27	
3.	Mean platelet volume (MPV)	Normal glucose tolerance (FBG < 5.6 mmol/L)	360	9.42	9.28	9.55	0.000
		Impaired fasting glucose (FBG between 5.6 & 6.9 mmol/L)	369	9.50	9.36	9.64	
		Newly diagnosed diabetes mellitus (FBG > 6.9 mmol/L)	91	9.69	9.42	9.96	
		Diagnosed diabetes mellitus on treatment	50	10.35	9.89	10.81	
4.	Platelet distribution width (PDW)	Normal glucose tolerance (FBG < 5.6 mmol/L)	360	13.95	13.95	13.95	0.339
		Impaired fasting glucose (FBG between 5.6 & 6.9 mmol/L)	369	13.88	13.88	13.88	
		Newly diagnosed diabetes mellitus (FBG > 6.9 mmol/L)	91	14.00	14.00	14.00	
		Diagnosed diabetes mellitus on treatment	50	14.01	14.01	14.01	

Figure 1: Univariate general linear model(GLM) keeping MPV as dependent variable, glycemic groups as fixed factors, gender as random factors and age as covariates [p=0.001]

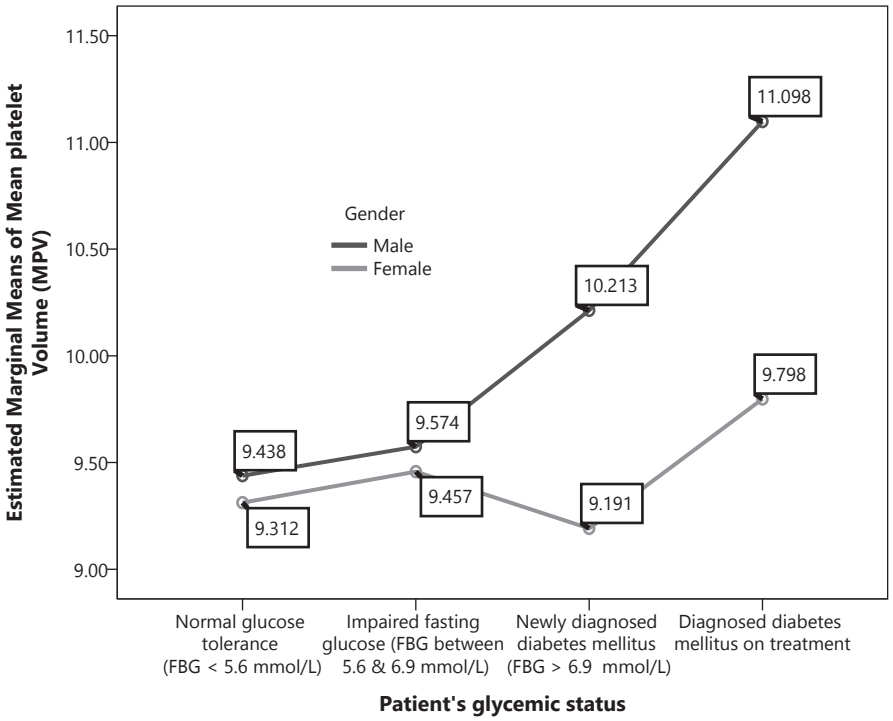


Figure 2: Differences in mean platelet volumes (MPV) among subjects diagnosed to have normoglycemia (n=30) and subjects with glucose deregulation [> 7.8 mmol/L] (n=47) based upon a 02 hour OGTT result [p=0.012]

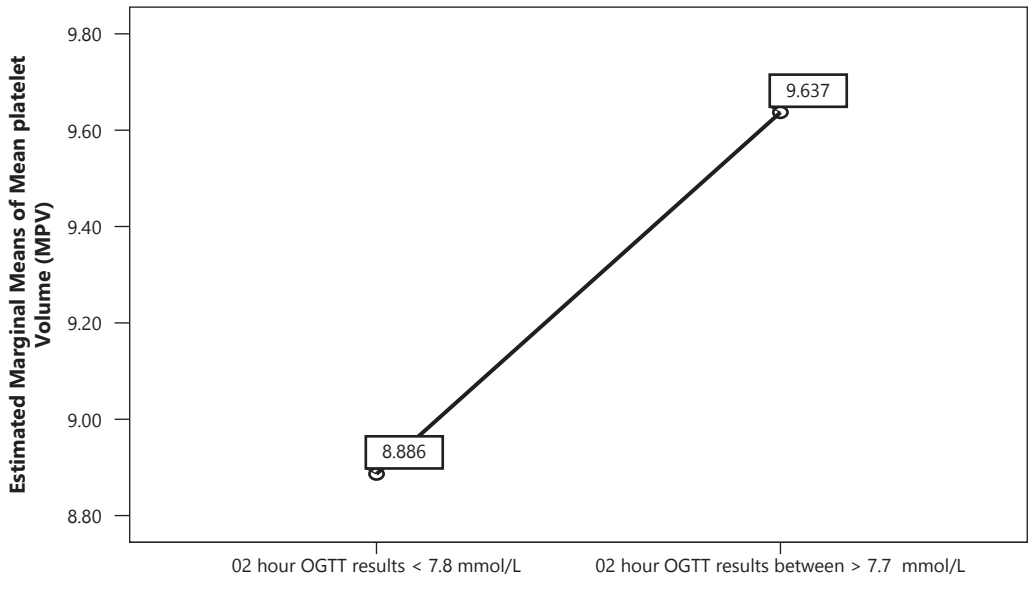
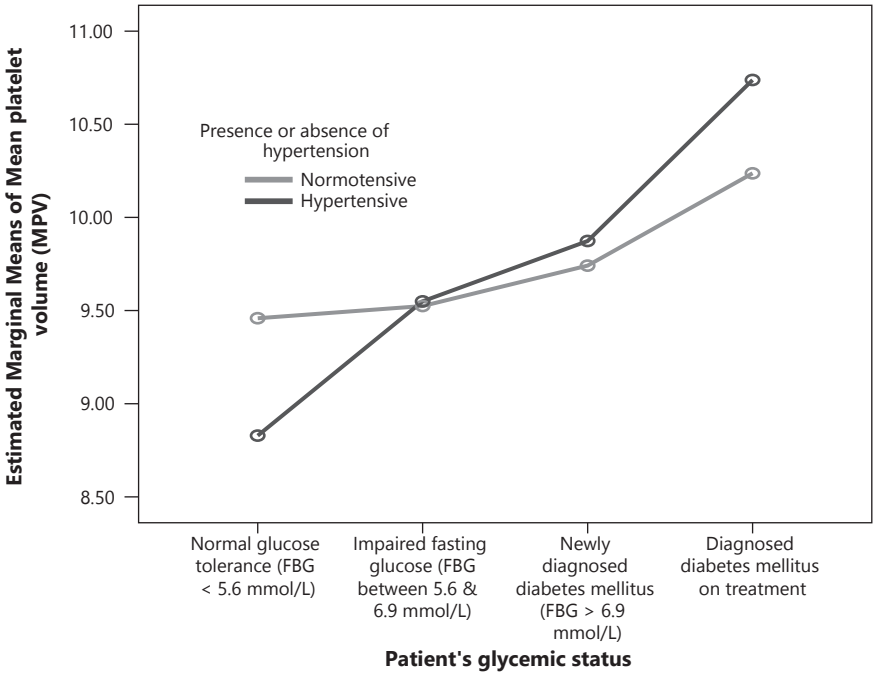


Figure 3: Differences in mean platelet volumes (MPV) between various grades of glycemia keeping hypertension as random factors in Univariate General Linear Model [p=0.107]



platecrit (PCT) are higher in subjects with hyperglycemia in comparison to normoglycemic subjects. This finding implies that hyperglycemia is associated with larger platelet volumes in human subjects. Our observation is supported by multiple studies as available in literature[15-17]. Considering hyperglycemia as an established risk factor, De Luca et al[10] have not depicted any significant differences for mean platelet volumes among patients with or without coronary artery disease, but confirm association with baseline hyperglycemia. Similarly, PROGRESS TRIAL (The Perindopril Protection Against Recurrent Stroke Study) did not show association with major coronary events, while the same study demonstrated a significant association with strokes[18]. The priority wise explanations leading to increase in mean platelet volumes in subjects with diabetes mellitus may include the following: Firstly, increased circulating glucose has earlier been demonstrated to cause structural changes in most body organs including the appearance of glycated hemoglobin, fructosamoine and advance glycation products, so the possibility of MPV rise could be natural consequence to hyperglycemia[19,20]. Thus we feel that platelets must also have undergone some modifications due to prevailing hyperglycemia. Secondly, the increase in platelet volumes could have resulted because of possible entry of glucose into the inside of platelets thus creating a pulling force on plasma fluid to move into platelets. In this regard Senen et al[21] have shown plasma viscosity to be related to mean platelet volumes. These changes in plasma viscosity may be the result of some acute phase reactants in plasma, as some authors have also described the association of mean platelet volumes with acute phase reactants like C-reactive proteins[22]. Finally, evidence is also there to demonstrate a rapid turnover of platelets in individuals who had certain cardiovascular risks, which could lead to appearance of early platelet forms which are larger than usual[23]. This modification thus, may appear in the apparent lab measured parameters by having increased overall platelet volumes (MPV) and also the relative percent increase in the platelet volumes (PCT). Another important aspect of our findings is the fact that the rise of MPV from normolgycemics to subjects having impaired fasting glucose and newly diagnosed diabetics remain minimal; but the change peaked in subjects with established diabetes with marked hyperglycemia. So whatever, the changes we may have observed are quite subtle initially and it may requires a long duration of marked hyperglycemia to become apparent in agreement with the possibility of associated damage to body tissues and associated complications[24]. In this connection Papanas et al[25] have demonstrated higher MPVs in diabetic subjects who had associated microvascular complications including retinopathy and microalbuminurea[4]. Similarly, literature review suggests a stronger link between post load hyperglycemia and major cardiovascular diseases and underlying diabetic complications. That could be one reason that individuals who demonstrated post load hyperglycemia simply demonstrated higher platelet volumes in our study.

Our study has observed minimal changes in platelet distribution width and platelet counts between subjects having various grades of glycemia. In this regard Jindal et al[26] have demonstrated PDW to be an indicator of microvascular complications in subjects with diabetes mellitus. However, others have not observed a significant association between PDW and coronary artery diseases[27]. The number of platelets and the platelet distribution width may simply signify a different dimension of platelet function, and may not be true reflection of acceleration in thrombotic process.

None of the platelet indices were having significant differences among subjects with and without hypertension in our study. Literature search reveal contrasting evidence in regard. Nadar et al[28] have demonstrated higher MPVs in hypertensives. Others segregating hypertensive subjects between dippers and non-dippers only showed higher MPV for the later variety[29]. While we did not find any matching reference to our finding, but literature search does suggest findings related to raised platelet volumes and thrombotic events to be as a consequence rather than a predictor or cause. [30] However, the authors do realize further research on this particular aspect by assessing the effect of platelet indices in newly diagnosed hypertensive's and with varying grades and types of hypertensions.

Certain limitations to our findings must be acknowledged: Firstly, the study focuses on Pakistani population who like the rest of the Asian population may have higher and different ethnic and racial differences as being highlighted in some studies. Secondly, we do acknowledge the limitations of a cross-sectional design where all possible confounders may not have controlled. However, considering the sample size we do feel that our findings may be relevant.

Our study may have enormous clinical implications due to the fact that mean platelet volume is a very simple and cost-effective modality, which once utilized in the light of our results can help can hint towards the underlying diabetes or its associated complications. Moreover, the study also provided an opportunity to a newer dimension of research which could lead to actual identification of causative targets for both diagnostic and therapeutic intervention.

CONCLUSION

Conclusion Mean platelet volume and platecrit increases across various grades of hyperglycemia. However, the changes become quite prominent in subjects having established diabetes. Post load hyperglycemia was more predictive of rises in mean platelet volumes.

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CONTRIBUTORS

SHK conceived the idea, did data collection and wrote the manuscript. SAA helped in data collection and writing up of manuscript. Both authors contributed significantly to the final manuscript.