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Can Platelet Large Cell Ratio and High-sensitivity C-reactive Protein be used as Predictive Markers for Vascular Complications in Type 2 Diabetes Mellitus?

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Abstract

Background and Aims: A large proportion of patients with diabetes mellitus suffer from preventable vascular angiopathies. Altered platelet structure and functions have been linked with these vascular complications. Hence, this study was undertaken to correlate platelet indices and high-sensitivity C-reactive protein (hs-CRP) levels with glycemic control and vascular complications to assess whether they can be used as predictive factors.

Materials and Methods: A total of 56 non-diabetics (control) and 145 diabetic patients were enrolled in this study. Criteria of fasting blood glucose ≥126 mg/dL/postprandial plasma glucose (2 h) levels >200 mg/dL/HbA1c ≥6.5 were considered. The diabetic group was further subdivided into diabetics without (82) and with complication (63) on the basis of clinical presentation, investigation, and examination. Platelet indices (mean platelet volume [MPV], platelet distribution width [PDW], and platelet large cell ratio [P-LCR]) were assessed on complete blood count analyzer. hs-CRP was done qualitatively and those samples which tested positive were assessed quantitatively.

Observation: All the three platelet indices assessed - MPV, PDW, and P-LCR were significantly higher in diabetics compared to non-diabetic group and increased with increasing HbA1c level. However, only P-LCR showed a significant difference between diabetics with and without complications ($P = 0.002$) and MPV showed a significant difference among all the subgroups when correlated with HbA1c ($P \leq 0.04$). For hs-CRP, the difference in the values was significant among the diabetics with and without complications ($P = 0.01$).

Conclusion: A continuous increase in the value of MPV, PDW, and P-LCR with decreasing glycemic control proves that in diabetics ongoing inflammation causes persistent generation of larger platelets with enhanced activity. P-LCR should be the indice of choice for predicting the possibility of future complication as in our study, it was the only parameter which showed significant difference between diabetics with and without complications.

Key words: Platelet indices, Thromboses, Inflammation, HbA1c, Diabetes.

Introduction

Diabetes mellitus (DM) is increasing in leaps and bounds and this pandemic disorder, causing large-scale morbidity and mortality, is a big economic burden, especially in poor countries. As of 2014, an estimated 387 million people had diabetes worldwide [1]. DM is characterized by varying degree of hyperglycemia accompanied with the biochemical alterations in carbohydrate, protein, and lipid metabolism. Deaths due to diabetes are increasing and there is a need to prevent these deaths by early diagnosis of impending complications. A large proportion of patients with Type 2 DM suffer from preventable vascular angiopathies which are categorized as macrovascular (coronary artery disease, peripheral arterial disease, and stroke) and microvascular (diabetic neuropathy, nephropathy, and retinopathy), with a 2–4-fold increase compared to non-diabetics (ND) [2]. Hence, there is a need to develop risk factor modifications and interventions to reduce the impact of such complications. Altered platelet structure and functions have been reported in diabetic patients and are linked with these vascular complications [3]. Identification of patients with abnormal platelets can easily be made during routine hematological analysis which is a simple, easy, and cost-effective tool that could prove to be a good indicator for timely intervention and prevention of the aforementioned complications. During the past few years, the role of highly sensitive C-reactive protein (hs-CRP) which is an acute phase protein secreted by the liver as well as by other tissues in response to any inflammatory condition has been reported as one of the most important pro-atherosclerotic mediators [4] and is now being widely used to predict the development of cardiac complications in ND [5] as well as patients of type 2 DM [6]. Hence, this study was undertaken to correlate platelet indices and hs-CRP levels with glycemic control and vascular complications with the vision that if found significant, they

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Table 1: Comparative evaluation of HbA1c and various platelet indices in controls, diabetics without complications, and diabetics with complications

Entity	Controls (A) (n=56)	Diabetics without complication (B) (n=82)	Diabetics with complication (C) (n=63)	P [A versus B]	P [A versus C]	P [B versus C]
HbA1c	5.08 (0.32)	8.56 (2.33)	9.23 (2.47)	0.0001 [S]	0.0001 [S]	[NS]
MPV	9.42 (1.03)	10.02 (1.22)	9.88 (1.21)	0.03 [S]	0.003 [S]	[NS]
PDW	12.4 (1.49)	15.03 (1.93)	14.36 (2.176)	0.0001 [S]	0.0001 [S]	[NS]
L-PCR	27.16 (6.89)	29.89 (8.09)	33.78 (6.7)	0.04 [S]	0.0001 [S]	0.002[S]

[S]: Significant, [NS]: Not significant, MPV: Mean platelet volume, PDW: Platelet distribution width, P-LCR: Platelet large cell ratio

Table 2: Platelet indices parameter with relation to HbA1c level

Entity	HbA1C (≤7%) [A]	HbA1C (7.1–8%) [B]	HbA1c (>8%) [C]	P [A versus B]	P [A versus C]	P [B versus C]
MPV	9.71 (1.23)	10.31 (1.32)	10.82 (1.04)	0.047 [S]	0.0001 [S]	0.04 [S]
PDW	14.2 (2.35)	14.32 (2.1)	14.52 (2.1)	[NS]	[NS]	[NS]
L-PCR	30.49 (7.65)	31.25 (7.48)	32.21 (7.61)	[NS]	[NS]	[NS]

[S]: Significant, [NS]: Not significant, MPV: Mean platelet volume, PDW: Platelet distribution width, P-LCR: Platelet large cell ratio

Table 3: Mean and standard deviation of hs-CRP in controls, diabetics without complications, and diabetics with complications along with degree of significance

Entity	Controls [A] (n=2)	Diabetics without complications [B] (n=3)	Diabetics with complications [C] (n=11)	P [A versus B]	P [A versus C]	P [B versus C]
hs-CRP	19.99 (5.83)	29.6 (4.76)	55.28 (28.1)	[NS]	0.03 [S]	0.01 [S]

[NS]: Not significant, [S]: Significant, hs-CRP: High-sensitivity C-reactive protein

could be included in the factors determining prognosis and outcome of diabetic patients.

Materials and Methods

Patient population and study design

The study was conducted in the department of pathology in collaboration with the department of medicine. The study was approved by the local ethical committee. Informed consent was taken from each individual at the time of inclusion in the study. 56 ND (control) and 145 diabetic patients were enrolled in this study. The diabetic group was further subdivided into diabetics without (82) and with complication (63) on the basis of clinical presentation, investigation, and examination.

Sample collection

Venous blood samples were collected in tubes with:

a. Dipotassium EDTA as anticoagulant and analyzed within 2 hours of venipuncture for platelet indices and HbA1c,

b. Sodium fluoride vial for fasting blood glucose and postprandial blood glucose (2 hours) and

c. In plain vial for hs-CRP.

Methods

Fasting and postprandial blood glucose (2 h)

Enzymatic determination of glucose was done in Selectra Pro M - Random Access analyzer using glucose oxidase and peroxidase method. For assigning our study population in the diabetic group, criteria of fasting blood glucose ≥7.0 mmol/L (126 mg/dL)/postprandial plasma glucose (2 h) levels ≥11.1 mmol/L (200 mg/dL) were considered.

Hemoglobin A1c

Percentage determination of hemoglobin A1c in human whole blood was performed using Bio-Rad D-10 TM dual program. The D-10 dual program is based on chromatographic separation of the analytes by ion-exchange high-performance liquid chromatography. Two-level calibration was used for quantification of the HbA1c values. A sample report and a chromatogram were generated for each sample. The HbA1C was calculated using an exponentially modified Gaussian algorithm that excluded the labile A1c and carbamylated peak areas from the A1c peak area. We considered criteria of HbA1c ≥6.5% according to the WHO 2016 for the diagnosis of DM.

Platelet indices

Platelet indices (mean platelet volume [MPV], platelet distribution width [PDW], and platelet large cell ratio [P-LCR]) were assessed by Medonic M-20 series auto loader analyzer which is based on impedance phenomenon. MPV was calculated by the formula = (plateletocrit [PCT] [%]/platelet count [PC] [×109/l]) ×105. PDW and P-LCR were analyzed from a histogram of platelet size distribution.

hs-CRP

a. Qualitative measurement - the CRP-latex agglutination test was used for the qualitative and semi-quantitative detection of CRP in human serum.

b. Quantitative measurement - the CRP-Turbilatex was used which is a quantitative turbidimetric test - latex particles coated with specific human anti-CRP are agglutinated when mixed with samples containing CRP which causes an absorbance change, dependent on the CRP concentration that can be quantified by comparison from a calibrator of known CRP concentration. The cutoff value was 6 ng/mm3.

Statistics

The data were put in a master chart in Excel Sheet and various parameters in different groups were compared. Chi-square test with or without Yates' correction and Student's unpaired "t" test were used as and where required. $P \leq 0.05$ was taken as critical level of statistical significance.

Observation

A total of 56 ND (controls) and 145 diabetic patients were enrolled in this study.

The diabetic group was further subdivided into diabetics with complication and diabetics without complication on the basis of clinical details and examination.
I. Control/ND group - 56 controls were included in this study. A cutoff value of HbA1C <5.7% and fasting blood glucose <100 mg/dl was used as criteria for choosing the control group so that even borderline diabetics were excluded from the study. The M:F ratio was 1.15:1.
ii. Diabetics (D) - 145 diabetic patients were included in this study. Value of HbA1C ≥6.5% or fasting blood glucose level ≥126 mg/dl or postprandial blood glucose level ≥200 mg/dl was taken as cutoff for inclusion in this group. Out of these 145, 82 (56.55%) had no associated complication on the basis of clinical details and other investigations, while the rest 63 (43.45%) had complications.
The M:F ratio in diabetics without and with complications was 1.8:1 and 1.6:1, respectively. Evaluation of HbA1c and platelet indices was done and compared among the three groups (Fig. 1) and analyzed for the degree of significance (Table 1). Whereas MPV and PDW were significantly higher in diabetics compared to ND group, they did not show any significant difference with regard to presence or absence of complications; P-LCR value was significantly higher in diabetics along with a significant difference seen between diabetics with complications and without complications (P = 0.002).

Platelet indices and their relation to HbA1c level

On the basis of the levels of glycosylated level (HbA1c), the diabetic patients were subclassified into three categories of mild (≤7%), moderate (7.1–8%), and severe (>8%). Various platelet parameters were compared among these categories (Table 2). Although all the three platelet indices, that is, MPV, PDW, and P-LCR, increased with increasing HbA1c level, significant difference among all the subgroups was seen with MPV only (P ≤ 0.04).

hs-CRP

CRP was performed in all the patients by latex agglutination method in which 16 cases turned out to be positive which were quantitatively analyzed by turbidimetric method. Out of 16, 11 were diabetics with complications, three were diabetics without complications, and two were ND (Control). Mean (SD) values in different groups were as shown in Table 3. The difference in the values was significant among the diabetics without and with complications (P =0.01).

Discussion

DM has now gained the status of a pandemic with India contributing a substantial number of cases. As per the 2015 data, we had 69.2 million people living with diabetes (8.7%) [7]. DM is a complex metabolic syndrome characterized by chronic hyperglycemia with associated complications chiefly arising out of micro- and macro-angiopathic changes. It is considered as a "prothrombotic state" with enhanced platelet reactivity. Inadequate glycemic control, protein glycation, and oxidative stress cause endothelial injury and platelet activation with altered platelet morphology and function leading to chronic complications in diabetics [8]. Several studies have proven that platelets with increased size contribute in pathogenesis of vascular complications [9, 10, 11, 12, 13], larger platelets being more active enzymatically and metabolically with higher thrombotic ability [14]. Increased platelet sensitivity is related to the enhanced release of contents from platelet granules which, in turn, lead to the making of a platelet volume gradient, increased platelet turnover rate, and reduction in survival of platelets [15]. Various platelet parameters help in identifying platelet proliferating activity such as PC, PCT, MPV, PDW, and P-LCR [16]. The present study was aimed to find out the relation between platelet indices (excluding PC and PCT) and hs-CRP levels with glycemic control and the presence of micro/macrovascular complications in diabetics. 56 ND (control) and 145 diabetic patients were enrolled in this study. Diabetic group was further subdivided into two, on the basis of the absence of complications (82) and the presence of complications (63). MPV can be used as a marker of production rate and platelet activation [17]. In our study, significantly higher values of MPV were observed in diabetics when compared to non-diabetic group. However, no significant difference could be observed with regard to the presence or absence of complications. Our findings were consistent with that of some earlier studies [14, 15, 18, 19, 20]. On the contrary, a few studies have reported a significant difference in MPV values of diabetics with and without complications [2, 16, 21, 22, 23], indicating that with ongoing thrombosis, newer platelets released in circulation are bigger, and hence, the MPV increases. PDW directly measures variability in platelet size, changes with platelet activation, and reflects the heterogeneity in platelet morphology [24]. In the present study, PDW value was significantly higher in diabetics as compared to controls, similar to earlier studies [4, 16, 19]. However, on comparison between diabetics with and without complications, no significant difference was found. Some past studies have shown significant difference between these two groups with respect to PDW [2, 4, 14, 16, 22]. High PDW value in diabetics results from the increased number of large platelet production and their activation.
P-LCR, defined as the percentage of platelets that exceed the normal value of platelet volume of 12 fL in the total PC, is used for monitoring platelet activity [16]. In the present study, P-LCR value was significantly higher in diabetics as compared to ND group. A significant difference was also observed in diabetics with and without complications. This was in accordance with Shilpi et al. [19] and Hong et al. [25]. However, some other workers [14, 16, 22] did not see any significant difference in relation to the presence or absence of complication in their respective studies. P-LCR was increased in this study probably due to ongoing microvascular phenomenon causing compensatory persistent generation of activated platelets. CRP is an acute phase reactant which is produced by hepatocytes. It is a well-known non-specific marker of inflammation and tissue damage. In 2003, the Centre for Disease Control and Prevention and the American Heart Association recommended patient stratification into three groups in cardiovascular disease risk assessment: Low risk (hs-CRP <1 mg/L), intermediate risk (hs-CRP 1–3 mg/L), and high risk (hs-CRP >3 g/L). They also concluded that hs-CRP was the analyte of choice to identify patients for primary prevention of cardiovascular disease [8]. The value of hs-CRP showed a significant difference among the diabetics with complications and controls as well as diabetics without complications subgroup. This was similar to a few earlier reports where higher CRP value in diabetics group with complications

has been reported [20, 26]. The increased CRP reactivity observed in diabetics with complication may be attributed to microvascular thrombosis caused by inflammatory process. We also analyzed our data on the basis of the level of glycosylated level (HbA1c) and subclassified the diabetic patients into three categories - mild (≤7%), moderate (7.1–8%), and severe (>8%). We compared the various parameters of platelet indices and hs-CRP levels among these categories. Among the platelet indices, significant difference among all the subgroups was seen with MPV only. Many authors also have reported similar findings [2, 27, 28], but Bhanukumar et al. [18] have stated that though MPV and PDW values are higher in patients of diabetes, they do not correlate with HbA1c levels. For PDW and P-LCR, in the present study, we found that their levels were slightly higher in patients with moderately increased HbA1c level that further increased in patients with markedly increased HbA1c level, but they failed to reach the statistical significance. However, Bhattacharjee et al. [21] and Demirtas et al. [26] have stated them to significantly change with changes in HbA1c levels. With regard to hs-CRP, difference was significant among diabetic patients with mildly and markedly raised HbA1c only. In a quite similar study, Shi et al. divided diabetic patients, according to their HbA1c levels, into three groups: HbA1c ≤9.32%, HbA1c >9.32 and ≤11.76%, and HbA1c >11.76%. They found that the level of hs-CRP was significantly different between all the three groups [29]. In another study, correlation between HbA1c and hs-CRP was done where patients with hs-CRP <1 mg/dl showed HbA1c values which were statistically different from those who had hs-CRP >1 mg/dl [30]. In the present study, patients showing reactivity for hs-CRP increased as the HbA1c control became poorer indicating that inflammatory process due to vascular thrombosis was more extensive in diabetics with poorly controlled HbA1c levels. Although various papers which have studied the role of platelet indices in predicting development of complications in diabetics have also taken PC and PCT into consideration, we have not taken these two parameters into account due to the high variability shown by these with regard to numerous environmental factors. In this study, we did not correlate the platelet indices with hs-CRP as due to financial constraints, quantitative estimation of it was done in only those 16 cases who showed positive results on qualitative estimation. The present study shows the hypotheses that in diabetics due to ongoing inflammation, there is persistent microthrombi formation, leading to platelets consumption and generation of larger platelets with enhanced activity, holds true. A continuous increase in the value of platelet indices, especially MPV, PDW, and P-LCR with decreasing glycemic control, emphasizes that their regular assessment may be very helpful in picking up those diabetics who are more likely to develop the vascular complications.

Since hs-CRP is considered as a very sensitive marker of vasculopathy, its correlation with individual platelet indices in future researches will help us to get a more clear vision. We would also wish to comment that out of MPV, PDW, and P-LCR, the latter should be the indice of choice for predicting the possibility of future complication as in our study, it was the only parameter which showed significant difference between diabetics with and without complications.

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