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Research paper

Differences in platelet indices between type-2 diabetes mellitus patients with and without microvascular complications at Teaching Hospital, Karapitiya

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Key words: type-2 diabetes mellitus, platelet indices, microvascular complications

Abstract

Type-2 Diabetes Mellitus (T2DM) is associated with a greater amount of morbidity and mortality due to microvascular and macrovascular complications. Involvement of platelets in vascular complications of diabetes is well known and changes in platelet indices have been identified as predictors of these vascular complications. This study was carried out to determine the changes in platelet indices in patients with T2DM. This was a comparative cross sectional study conducted at Teaching Hospital, Karapitiya. Study subjects included T2DM patients with microvascular complications as study group and T2DM patients without any vascular complications as comparison group. For each group age and sex matched 52 subjects were recruited. Subjects with microvascular complications had mean platelet volume (MPV) of 10.62±0.49fL, platelet distribution width (PDW) of 12.23±1.12fL, plateletcrit (PCT) of 0.28±0.05% and platelet large cell ratio (PLCR) of 29.44±4. Subjects without any vascular complications had MPV of 9.38±0.40fL, PDW of 9.9±1.14fL, PCT of 0.26±0.05% and PLCR of 19.67±3.1. Values of MPV, PDW and PLCR were significantly higher in patients with diabetic microvascular complications (p<.001). There was no significant difference in PCT between the two groups. In comparison of platelet indices according to glycemic control, a significantly higher MPV, PDW and PLCR values were observed in patients with HbA1c of >7% (p<.001). A significant association was present among the MPV, PDW and PLCR values when fasting plasma glucose (FPG) was >130mg/dL (p<.001). There was a direct relationship with abnormal platelet indices and microvascular complications of T2DM. Further studies are recommended to ascertain causative relationship of changes in platelet indices and diabetic microvascular complications.

Introduction

Type-2 Diabetes Mellitus (T2DM) is a common chronic metabolic disorder affecting 463 million people in the world with a prevalence of 8.7% among Sri Lankan adults[1]. Diabetes mellitus (DM) is associated with a significant morbidity and mortality and estimated to be the 7th leading cause of death by 2030[2].

DM is invariably associated with complications broadly categorised as microvascular and macrovascular complications. Most diabetic patients develop these complications during the course of their illness.

Retinopathy, nephropathy and neuropathy are microvascular complications. They are unique to DM affecting capillaries and arterioles of retina, kidneys and nerves[3]. Ischaemic heart disease, cerebrovascular disease and peripheral arterial disease are macrovascular complications.

According to Sri Lankan data[4], prevalence of retinopathy, neuropathy and nephropathy were 26.1%, 62.6% and 50.8% respectively in T2DM.

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Chronic hyperglycaemia affects vascular endothelium both directly and indirectly resulting in vascular complications. Both macro and microvascular complications are associated with defects in platelets and neurovascular units[5].

Platelets play a vital role in haemostasis. Several studies revealed that platelets contribute to development of DM related vascular complications[5,6,7]. Enhanced coagulation, compromised fibrinolysis and endothelial dysfunction lead to platelet hyper-reactivity. Hyperactive platelets play a crucial role in pathophysiology of thrombosis causing complications[5,6,7].

Even though role of platelets in macrovascular complications are evident by many studies, research related to effect of platelets on microvascular complications are few. Some studies have revealed larger platelets with higher MPV are more reactive and associated with an increased risk of complications[8,13].

Automated full blood count analysers provide platelet parameters including mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), platelet large cell ratio (PLCR) and platelet large cell count (PLCC).

MPV denotes average size of platelets. PDW represents platelet size distribution. PCT reflects total platelet mass. PLCR is the ratio of larger platelet (MPV >12fL) count to total platelet count which is expressed as a percentage.

Full blood count (FBC) is performed in standard care setting in every diabetic patient. It provides all these platelet parameters. Fasting plasma glucose (FPG) and glycated haemoglobin (HbA1c) are regularly performed in diabetic patients as glycaemic control measures.

Many studies have shown a significantly higher MPV in diabetic patients compared to healthy controls. A significantly higher MPV was evident in diabetics with complications compared to diabetics without complications.

A study done in India[11] revealed a significantly higher MPV in diabetics compared to non-diabetic people (p=0.023). They noticed a higher MPV and PDW in diabetics showing poor glycemic control with HbA1c of >7%.

B F Zuberi, et al.[8] revealed a significantly higher MPV in diabetic patients when compared to non-diabetics.

Rajas S Walinkar, et al.[9] demonstrated a significantly higher MPV, PDW and PLCR in diabetic patients with microvascular complications compared to diabetics without microvascular complications ($p < 0.0001$).

Archana Buch, et al.[10] depicted a significant difference in MPV and PDW in diabetic patients compared to non-diabetic controls. They have concluded that MPV and PDW are predictors of diabetic vascular complications.

In contrast, Akinbami Akinsegun, et al.[12] did not find a significant difference in MPV between diabetics and healthy controls.

Dindar S, et al.[13] noticed a positive correlation of MPV with FPG ($p=0.03$) and HbA1c ($p < 0.001$).

A study done in Bosnia and Herzegovina[14] depicted a significantly higher MPV in diabetic patients who had an HbA1c of >7% ($p < 0.0005$). Further, at the best cut off value of 9.95fL, MPV showed a sensitivity of 82% and specificity of 54.5% in predicting deterioration of glycemic control in diabetic patients.

No studies were found regarding evaluation of platelet indices in T2DM patients in Sri Lanka.

If there is an association with microvascular complications and platelet indices, it would guide clinicians for early prediction and take timely interventions during patient follow-up. This will be helpful in reducing diabetes related morbidity and mortality.

Therefore this study was carried out to evaluate differences of platelet indices in T2DM patients with and without microvascular complications at Teaching Hospital, Karapitiya.

Methodology

This was a comparative cross sectional study in T2DM patients and comprised of two groups. Diabetic patients with microvascular complications were considered as study group and diabetic patients without having any complications were the comparison group.

Patients aged between 30-70 years with confirmed T2DM having microvascular complications followed up at endocrine and general medical clinics at Teaching Hospital, Karapitiya from 15.07.2020-15.11.2020 were enrolled upon receipt of consent until required sample size (52) was achieved as study group.

For comparison group, age matched T2DM patients without having any vascular complications followed up at endocrine and general medical clinics at Teaching Hospital, Karapitiya from 15.07.2020-15.11.2020 were enrolled upon receipt of consent until required sample size (52) was achieved.

Diabetic patients having macrovascular complications, patients with abnormal platelet number or function and patients having anaemia according to WHO recommendations were excluded from our study.

Sample size was calculated using the following formula[16].

$$n = \frac{(u+v)^2 \quad (S_1^2 + S_2^2)}{(x_1 - x_2)^2}$$

Mean and standard deviations (SD) were extracted from a similar study conducted by Rajas S Walinjkar, et al[8].

According to it, minimum sample size calculated for one group was 45. We recruited a similar number of patients as study group (n=52) and comparison group (n=52).

Data collection was done using an interviewer administered questionnaire and tracing medical records of study population into a data collection sheet.

Diabetic retinopathy was diagnosed by standard ophthalmological examination performed by a consultant ophthalmologist.

Presence of microalbuminuria (30-299mg/day) or macroalbuminuria (≥300mg/day) was diagnosed as nephropathy.

Presence of neuropathy was determined by clinical examination performed by a consultant physician or endocrinologist or supported by nerve conduction studies.

T2DM patients who had any one or more of documented microvascular complications according to above criteria were identified as study group.

T2DM patients who did not have any vascular complications were identified as comparison group.

All FBC samples were analysed by same analyser (Sysmex XN - 1000). Internal quality control (IQC) was assured in analyser by running IQC samples daily.

Blood film routinely prepared for verification of each FBC sample was examined for the presence of platelet clumps, platelet satellitism, fragmented red cells and fibrin strands. Those samples bearing such abnormalities were excluded from study.

Data analysis

Data was analysed using statistical package for social sciences (SPSS) version 20. Descriptive statistics of continuous variables were expressed as mean and SD. Categorical variables were shown with frequency and percentages. Platelet indices in patients with T2DM having microvascular complications were compared with those of diabetics without having any complications. Results were described entirely in relation to study group and comparison group.

Variables showing normal distribution were further analysed using independent t-test. Other variables were analysed using Mann-Whitney U test and

Wilcoxon sign test. P values were obtained using Chi-square test. Receiver operating characteristic curve was drawn to calculate optimum cut-off values for MPV, PDW and PLCR. All hypotheses were tested at 95% confidence interval.

Ethical clearance was obtained from Ethical Review Committee of Faculty of Medicine, University of Ruhuna.

Results

The mean age of individuals in study group was 56.06±8.03 years. In comparison group, it was 55.37±8.11 years. There was no statistically significant difference in distribution of age, gender, ethnicity, marital status and habit of smoking between two groups.

Duration of diabetes since diagnosis was significantly higher in study group. (10.26±5.34 in the study group and 6.42±3.99 in the comparison group, p<0.001)

Twenty two patients (42.3%) in the study group were on antiplatelets. In the comparison group, 27 individuals (51.9%) were on antiplatelet drugs.

Comparison of platelet indices in study sample

In diabetics with microvascular complications, mean MPV was 10.62±0.49fL and it was 9.38±0.40fL in diabetics without complications showing a significant association (p<.001). Mean PDW was 12.23±1.12fL in study group and 9.9±1.14fL in comparison group with a significant relationship (p<.001). Mean PLCR value was 29.44±4.5 in study group and 19.67±3.1 in comparison group with a significant association (p<.001). Mean PCT values was 0.28±0.05% in study group and 0.26±0.05% in comparison group with no significant association. Mean platelet count was 269.84±49.03×10^9/L in study group and 285.42±46.57×10^9/L in comparison group with no significant association.

Comparison of platelet indices with glycemic control

Whole study sample was divided into two subgroups according to glycemic control. Glycemic control was assessed by mean FPG of last 3 months and latest HbA1c value. There was a significant association among MPV, PDW and PLCR parameters with HbA1c (P<.001) and FPG (P<.001).

All the subjects in our study were divided into two sub groups according to duration of DM since

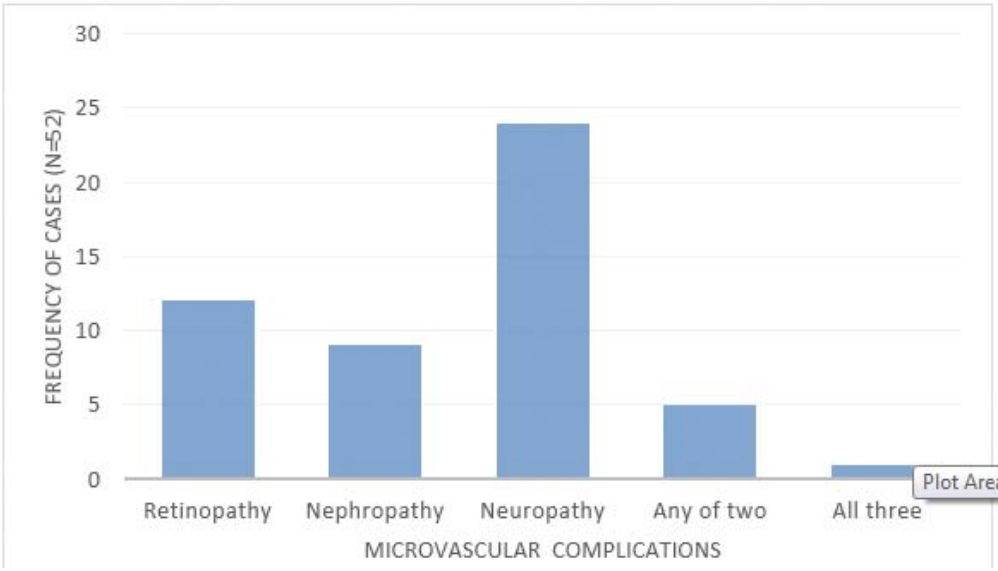

Figure 1. Distribution of microvascular complications in diabetic patients (study group n=52).

diagnosis. Those with disease duration >5 years were categorised into one group and the other group consisted of diabetics with disease duration <5 years.

Patient group of DM<5 years showed mean MPV of 9.75±0.69fL, mean PDW of 10.72±1.71fL, mean PCT of 0.28±0.05% and mean PLCR of 22.49±5.57. Patient group of DM>5 years had mean MPV of 10.11±0.78fL, mean PDW of 11.27±1.53fL, mean PCT of 0.27±0.05fL and mean PLCR of 25.41±6.35. A significantly higher MPV (p=0.017), PCT (p= 0.05) and PLCR (p=0.02) were noted with disease duration of >5 years.

There was no significant association with platelet indices and number of microvascular complications.

Receiver operating characteristic (ROC) curve was generated upon MPV, PLCR and PDW and presence of microvascular complications.

At the cut off of 9.95fL, it showed a sensitivity of 98.1% and specificity of 99.9%.

MPV more than 9.95fL was highly suggestive of presence of microvascular complications.

Table1. Comparison of platelet indices among diabetic patients with microvascular complications (Study group) and without complications (comparison group)

Platelet indices	Frequency (%)		p value
	Cases (n=52)	Control (n=52)	
MPV(fL)	10.62±0.49	9.38±0.40	<0.001
PCT (%)	0.28±0.05	0.26±0.05	0.18
PDW (fL)	12.23±1.12	9.9±1.14	<0.001
PLCR	29.44±4.5	19.67±3.1	<0.001
Platelet count ($\times 10^9$/L)	269.84±49.03	285.42±46.57	0.11

Table 2. Comparison of platelet parameters according to the HbA1c level within study sample

Sample (n=104)

Parameters	Mean MPV (fL)	Mean PDW (fL)	Mean PCT (%)	Mean PLCR
Group with HbA1C <7%	9.69±0.57	10.47±1.41	0.27±0.05	21.91±4.58
Group with HbA1C >7%	10.29±0.83	11.69±1.56	0.28±0.06	26.96±6.64
p value	<0.001	<0.001	0.328	<0.001

Table 3. Comparison of platelet parameters according to FPG level within study sample

Sample (n=104)

Parameters	Mean MPV (fL)	Mean PDW (fL)	Mean PCT (%)	Mean PLCR
Group with FPG <130	9.58±0.76	16.20±1.06	0.266±0.050	21.18±4.45
Group with FPG <130	10.44±0.73	12.01±1.55	0.28±0.053	27.89±6.01
p value	<0.001	<0.001	0.165	<0.001

Table 4. Summary of the sensitivity, specificity at its optimum cut off value per each platelet parameter

Parameter	Area under the curve (95% CI)	Cut off value	Sensitivity (%)	Specificity
MPV	.995 (.983-1.00)	9.95 fL	98.1%	99.9%
PLCR	.973 (.944-1.00)	22.25	96.1%	76.9%
PDW	.949 (.904- .995)	10.35 fL	96.1%	75.0%

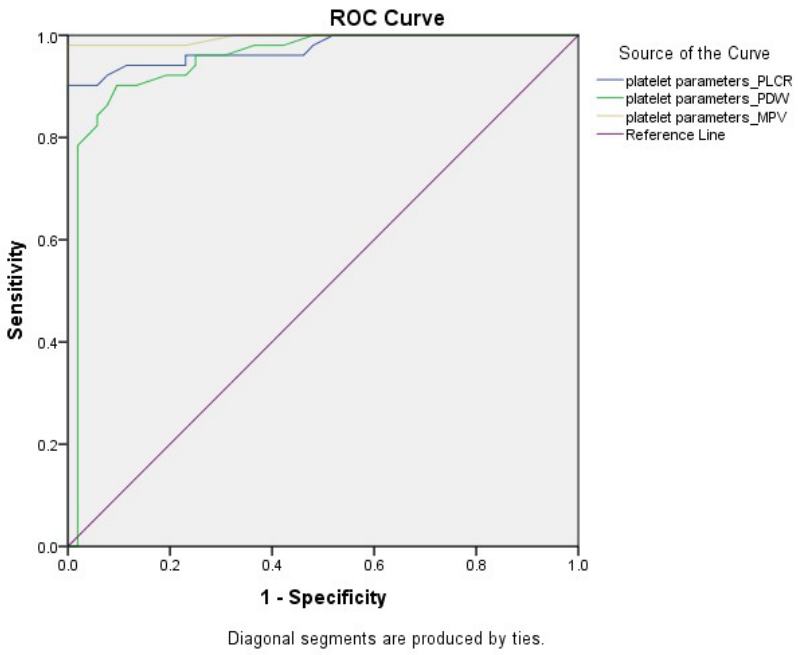

Figure 2. Receiver operating characteristic (ROC) by MPV, PLCR and PDW.

Discussion

Diabetic microvascular complications are thought to be associated with increased platelet activity. Platelet activation leads to morphological changes in platelets affecting their size and distribution. For early prediction of complications, attempts are being made to detect usefulness of platelet indices. With simple availability of these indices, this will be of tremendous advantage[9,10].

We analysed 104 diabetic patients with microvascular complications (n=52, study group) and without any vascular complications (n=52, comparison group). There was no significant difference in socio-demographic data between two groups. Mean duration of diabetes in study group was significantly higher than comparison group (10.26±5.34, 6.42±3.99, p<0.001 respectively). This can be explained by the natural course of DM where patients develop complications with time during the course of their illness.

MPV, PDW and PLCR were significantly higher in study group (p<0.001). There was no significant association between PCT and microvascular complications. These findings were consistent with results of a study done by Rajas S Walinjkar, et al.[9].

The whole study sample was divided into two subgroups according to mean FPG and HbA1c levels. FPG 130 mg/dL and HbA1c 7% were taken as cutoff points for glycemic control as per the standard clinical practice. We noticed a significantly higher MPV, PDW and PLCR values in diabetic with HbA1c of >7% (p<001). A study done in India reported a significantly high MPV and PDW values in diabetic subjects with HbA1c >7%[11]. In our study, FPG >130mg/dL was significantly associated with higher mean MPV, PDW and PLCR values (p<0.001). It emphasizes the importance of assessment of glycemic control when platelet indices are high in patients with DM.

The MPV (p=0.017) and PLCR (p=0.02) values were significantly higher when disease lasted beyond 5 years. A study done in India showed a significantly high MPV in patients with diabetes for >10 years[11]. However, these findings were in contrast to findings of Kodiatte, et al.

ROC curve was generated for presence of microvascular complications versus MPV showing that MPV more than 9.95fL has a sensitivity of 98.1% and a specificity of 99.9% for prediction of microvascular complications in patients with DM. This fact would be much helpful to identify such complications. Anyhow it may need further extensive research before claiming as such.

Our study didn't show a relationship with platelet indices and number of microvascular complications (p>0.05).

Conclusions

Platelet indices (MPV, PDW, PLCR) showed significantly higher values in diabetic patients with microvascular complications when compared to those without complications (p<0.001). Statistically significant associations were noted between platelet indices (MPV, PDW, PLCR) and glycemic control when HbA1c was >7% (p<0.001) and FPG was >130mg/dL (p<0.05). Significantly higher MPV and PLCR values were observed when duration of diabetes exceeded 5 years (p<0.05). No significant association was noted between PCT and microvascular complications. MPV of more than 9.95fL showed a sensitivity of 98.1% and a specificity of 99.9% for prediction of microvascular complications in patients with DM.

There was a direct relationship with abnormal platelet indices and microvascular complications in diabetic patients. We recommend assessment of platelet indices in parallel to routine glycemic control assessment in patients with DM as this can predict possible microvascular complications. However, causative or predictive association of platelet indices and microvascular complications in DM need further studies with larger samples drawn from community with multiple variables.

Limitations

External quality assurance was not performed for platelet indices in government sector laboratories of Sri Lanka at the time of data collection.

Authorship

Dr. V.N.U. Gamage wrote the research proposal, conducted data collection, performed data analysis and wrote the manuscript. Dr. M.G.M. Mohotti and Dr. K.A.C. Wickramaratne corrected the proposal, supervised and corrected the manuscript. We believe that manuscript represents honest work and we take full responsibility of the published material.

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Authors declare that there are no conflicts of interest.

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