

Platelet Indices and Basic Coagulation Profile in Haemodialysis Type 2 Diabetic Patients in Rural Population

H. PAHIM¹, SUPREETHA MEGALAMANE²

ABSTRACT

Introduction: Diabetes mellitus is rapidly growing and almost becoming epidemic, with currently 285 million people with diabetes worldwide. Chronic Kidney Disease (CKD) is evolving to be an important global disease, with diabetes playing a pivotal role behind this rapid outburst of CKD incidence.

Aim: To evaluate platelet indices and to study the coagulation profile in haemodialysis patients having Type 2 diabetes with CKD and diabetic patients without CKD.

Materials and Methods: This prospective study was conducted from March to December 2016 for 10 months. Two groups of patients were recruited. The first group (study group) consisted of 80 patients clinically diagnosed as CKD with Type 2 diabetes who were on haemodialysis treatment. The second group (controls) consisted of 80 cases diagnosed with Type 2 diabetes but without CKD, coming for routine checkups.

Results: Male predominance was seen in both groups. The red blood cells and leukocyte counts were within the reference values and showed no statistical difference between the groups. Haemoglobin was slightly lowered in study group than controls. Although, platelet counts were similar between the groups, there was an increased in Mean Platelet Volume (MPV) in study group $9.84 \pm 2.28\%$ and controls $6.59 \pm 1.69\%$ with p-value 0.002 significant. Also, increased PDW in patient's $17.93 \pm 2.79\%$ and controls $15.15 \pm 1.39\%$ with p-value 0.005 significant. Prothrombin Time (PT) and Activated Thromboplastin Time (APTT) both were increased in patients.

Conclusion: Our study concluded that platelet indices play a crucial role in patients having diabetes with CKD. Basic coagulation profile aids in predicting the long term complications in such patients. Haemodialysis, in these patients have more advantageous effect with few preventable factors, which can be overcome with monitoring platelet indices and coagulation profile.

Keywords: Chronic kidney disease, Diabetes mellitus, Diabetic platelets

INTRODUCTION

The prevalence of diabetes mellitus is drastically increasing worldwide and is approaching to epidemic proportions. Currently, 285 million people with diabetes are estimated around the world [1].

The natural history of diabetes is characterized by the variable occurrence and severity of microvascular and macrovascular complications. End Stage Renal Disease (ESRD) is an extreme manifestation of diabetic nephropathy, a microvascular complication of the disease and has increased risk for the appearance and progression of other microvascular complications, including retinopathy and neuropathy [2].

CKD is going to be established as an important chronic global disease where diabetes plays a very important key factor in its evolution. In India, both healthcare and economy will be affected by this increase trend in near future [3].

Recently, in one Indian population based survey report it was stated that age adjusted incidence rate of ESRD is 229 million. Because of lack of resources only 10% ESRD patients in India are benefited any Renal Replacement Therapy (RRT) [3]. Gold standard treatment of RRT for diabetes and ESRD is renal transplantation, not many patients can afford the treatment in India [4,5].

In CKD both bleeding and thrombotic complications are observed mainly because of disturbed balance between pro and anti haemostatic factors, leading to high morbidity and mortality [6].

Diabetes mellitus is associated with increased complications due to variety of abnormalities reported in diabetic platelets [7]. These diabetic platelets, in response to stimulating agents and spontaneous can exhibit increased adhesiveness and exaggerated aggregation phenomenon [8]. In Western population various studies have proven on platelet activation and its association with diabetes,

however very limited studies on Asian Indian populations [9]. Hence, it is necessary to commence such studies for future prospectives.

Substantial activation of diabetic platelets can occur in course of haemodialysis which may be due to exposure of blood to the roller pump segment and microbubbles in the haemodialyser. Hence, dialysis membranes quality plays a vital role in platelets activation [10].

Many studies from different parts of the world revealed that diabetes is a key factor for mortality in ESRD patients [5]. Thus, this study was conducted to evaluate the platelets indices in patients on haemodialysis having Type 2 diabetes with CKD as compared to diabetic patients without CKD. Bleeding and thrombotic complications are high possibility, so we also assess the basic coagulation profile.

MATERIALS AND METHODS

This prospective study was conducted in the Department of Pathology at RL Jalappa Hospital and Research Centre, Kolar, Karnataka, India, between the period of March to December 2016 for 10 months duration.

Two groups of patients were recruited. The first group (study group) consisting of 80 patients clinically diagnosed with Type 2 diabetes with CKD and receiving haemodialysis. Diagnosis of CKD was according to National Kidney Foundation, KDOQI CKD guidelines 2002 [11].

The second group (control group) consisting of 80 patients having Type 2 diabetes but without CKD, and visited for routine checkups. Diagnosis of Type 2 diabetes was based on the WHO criteria-Fasting plasma glucose ≥ 126 mg/dL and 2 hour post glucose ≥ 200 mg/dL [12]. Blood urea and serum creatinine were normal.

Informed consent was taken from all the participants (both study and control groups). Institutional ethical clearance was obtained prior to the study.

Patients with clinical signs of infection, malignancy, primary haemostatic disorders, treatment, use of antiplatelet agents (except aspirin) such as clopidogrel, dipyridamole or non-steroidal anti-inflammatory drugs and the inability to provide informed consent were excluded from the study.

Sample Collection

For study group: Since we wanted to analyse the effect of haemodialysis on haematological parameters and coagulation profile we collected two samples. Samples were collected from the patients haemodialysis, before starting heparinization and immediately after the procedure.

About 4-5 ml of venous blood was collected in 2 different test tubes containing sodium citrate for coagulation profile (PT, APTT) and EDTA for complete blood counts and platelet indices.

For control group: About 4-5 ml of venous blood was collected in two different test tubes containing sodium citrate for coagulation profile (PT, APTT) and EDTA for complete blood counts and platelet indices.

Complete Blood Count and Platelet Indices

RBC, WBC and platelet indices were estimated automatically by the ALERE H560 coulter method using EDTA anticoagulant blood samples.

Coagulation Study

PT, APTT values were measured automatically on STAGOSTART 4 instruments using 3.2% sodium citrate anticoagulant blood samples.

STATISTICAL ANALYSIS

The data were analysed using SPSS version 22. We applied descriptive statistics and exploratory data analysis to obtain means and standard deviations. For the qualitative variables, Chi-square test was performed. Independent t-test was used to test the difference between means. To verify the association between fasting blood glucose and platelet parameters, we applied Pearson's correlation, considering a statistically significant result when p-value < 0.05 and a strong correlation when $r > 0.6$.

RESULTS

Based on selection criteria described, 80 patients were considered in the study group having Type 2 diabetes with CKD (72 male and 8 female) and 80 patients having diabetes without CKD (52 male and 28 female) were included as control group. Male predominance was seen in both groups. Age was homogeneous in both the groups, mean age ranged from 51.90 ± 7.629 years.

Comparison between Study Group and Control Group

The red blood cells and leukocyte counts were within the reference values and showed no statistical difference between the groups. Haemoglobin was slightly lowered in study group (mean 8.77 ± 1.01) than controls (mean 10.7 ± 1.27). Haematocrit value showed mild reduction in study group (mean 25.5 ± 3.54) than controls (mean 27.12 ± 3.59) and p-value less than 0.0005.

Although, platelet counts were similar between the groups, there was an increase in platelet indices mainly MPV and Platelet Distribution Width (PDW) with significant p-value 0.002 and 0.005 respectively in study group.

PT and APTT both were increased in study group with significant p-value of 0.002.

Comparison between Pre Haemodialysis and Post Haemodialysis Sessions

The red blood cells and leucocyte counts showed no changes in study group between pre and post haemodialysis sessions. There

was improvement in haemoglobin levels and haematocrit readings in study group post haemodialysis sessions. Platelet count improved but MPV and PDW values were slightly decreased with p-value < 0.005 significantly in MPV values [Table/Fig-1].

PT showed not much difference whereas APTT values were higher compare to post dialysis (mean 52 ± 32.21) with p-value < 0.008 [Table/Fig-2].

Correlation of Fasting Blood Glucose and Platelet Parameters in Study and Control Groups

We observed a positive correlation with MPV (p-value < 0.005) and PDW (p-value < 0.005) in study group, indicating that higher fasting glucose levels tended to present higher values of MPV and PDW. Correlation was strong ($r > 0.60$) in MPV ($r=0.74$), however the correlation was weak in PDW ($r=0.49$). In control group, Pearson's correlation was not significant [Table/Fig-3].

Parameters	Pre Haemodialysis Phase (Mean±SD)	Post Haemodialysis Phase (Mean±SD)	p-value
Platelet Counts ($\times 10^9/\mu\text{L}$)	186.55±657.25	197.85±682.63	0.127
Plateletcrit (%)	0.107±0.04	0.197±0.26	0.145
Mean Platelet Volume (fL)	9.84±2.28	8.86±2.21	<0.005
Platelet Distribution Width (fL)	17.93±2.79	17.96±5.77	0.981

[Table/Fig-1]: Platelet indices comparison between pre haemodialysis and post haemodialysis in patients with diabetic CKD.

Parameters	Pre Haemodialysis Phase (Mean±SD)	Post Haemodialysis Phase (Mean±SD)	p-value
Prothrombin Time (in seconds)	19.73±4.29	19.75±4.26	0.975
Activated Partial Thromboplastin Time (in seconds)	49.18±29.9	52.01±32.21	0.008
INR	2.09±2.81	1.45±0.31	0.319

[Table/Fig-2]: Basic coagulation parameters in pre and post haemodialysis patients with diabetic CKD.

Parameters	Study Group		Control Group	
	Correlation (r-value)	p-value	Correlation (r-value)	p-value
Platelet Counts	0.31	0.039	-0.027	0.908
Mean Platelet Volume	0.74	0.002	-0.213	0.604
Platelet Distribution Width	0.49	<0.005	0.127	0.593
Plateletcrit	-0.69	0.145	0.025	0.428

[Table/Fig-3]: Correlation of fasting blood glucose levels with platelet indices in study and control groups.

DISCUSSION

World Health Organization in 2011 estimated that worldwide diabetes is prevalent in approximately 346 million people [13,14]. Diabetes is a complex metabolic syndrome featured by chronic sustained hyperglycaemia resulting in endothelial dysfunctions and vascular complications predominantly affecting organs like kidneys, peripheral nervous system and ocular organ [13,15,16].

In diabetics various mechanisms contribute to platelet dysfunction, affecting the adhesion, activation and also aggregation. Impact of Hyperglycaemia state on platelets effects calcium homeostasis and ultimately leads to cytoskeleton abnormalities and increased secretion of pro-aggregant factors, thus leading to athero-thrombotic changes [13,15,17-20].

Infact, several authors have analysed and proved that platelet changes are associated with diabetes. Furthermore, the wide

use of electronic analysers in various laboratories has allowed the quantification of platelets indices, which may reflect the functionality of these cells at affordable costs [19,21,22]. Among platelet indices, MPV and PDW stand out due to their involvement in development of thromboembolic complications [19,22,23].

The most significant findings in our study were an increase in MPV and PDW and we observed positive correlation between with these platelet indices with fasting glucose levels in patients with diabetic chronic renal diseases. Thus, poor glycaemic control can be important causation factor for endothelial dysfunction which can be associated with long term complications.

In our study, we observed that MPV was significantly higher in diabetics CKD (p-value 0.002) patients than control groups who were known diabetics, which was also supported by other studies [15,19]. Many studies have proven that an increased MPV are reflected by large circulating platelets and probably an independent risk factor for development of complications in diabetics [19].

Several studies have suggested that patients with diabetes have increased MPV when compared with non diabetic and, among the diabetics, those with vascular complications presented higher MPV values [19,21,22,24-28]. Some studies showed that MPV was significantly higher in diabetics patients with vascular complications than in diabetics without complications, similar documentation was done in our study [24,28-30].

In this present study PDW was higher in study group than controls, which was also show by Dalamaga M et al., [21]. The activated platelets differ in size from non activated ones mainly due to a change from a discoid to a spherical shape and pseudopodia formation, leading to a alterations in the PDW, as observed by Vagdatli E et al., [31]. In addition, significant differences were found in PDW parameter in diabetic patients with complications when compared with diabetics without complications, corroborating the results of Jindal S et al., [25]. These findings can be attributed to the accelerated production of platelets in patients with diabetics [19].

Vagdatli E et al., observed that PDW is a more specific diagnostic marker of platelet activation than MPV because it does not influenced by platelet swelling during single platelet distension. Thus combined use of MPV and PDW could predict activation of coagulation in a simple and affordable manner [19,31,32].

There are a few reports in the literature on Plateletcrit (PCT), and in the present study, we found no significant difference in the studied groups. Diabetic platelets are usually larger and more reactive thus platelet mass increase ultimately increases PCT [19,32,33] which was not seen in our study.

Hyperglycaemia is the diagnostic hallmark in diabetes. They are associated with hypercoagulable state, which can lead to long term complications [18,21,33-35]. This was explained by positive correlations which were found between fasting blood glucose levels with MPV and PDW in study group in our study. In our study there was strong correlation observed between fasting blood glucose and MPV, $r=0.74$ where strong association will be considered where r -value more than 0.60. With PDW moderate association was observed since, r -value was 0.49 which is less than 0.60. Platelet count showed weak association with fasting blood glucose where r -value was 0.30, which was consistent with various studies [18,19,21,33,35]. In our observation PCT showed negative association which was contrast finding with others [19,21,32,33,35]. Alterations in MPV and PDW were reflected by poor glycaemic control which may be due to osmotic effect caused from increased glucose levels and its metabolites in blood [19,21].

In our study, coagulation parameters were prolonged in patients than control. Monitoring PT and APTT levels help in determining the risk of development of bleeding complications [15,36].

In present study, the effect of haemodialysis on platelet indices was investigated by evaluating pre haemodialysis and post

haemodialysis sessions in study group. Studies have shown that dialysis partially corrects platelet dysfunction associated with CKD, but does not eliminate the risk of haemorrhage. Platelet-dependent fibrin clot formation, which is defective in these patients, improves with haemodialysis. This may contribute to the improved platelet function seen after dialysis [37,38]. In this present study platelet count and other platelet indices were improved, similar findings seen in other studies [15,19,21,37,38].

Recently, few studies have shown that substantial activation of platelet can occur in the course of haemodialysis. Platelet surface markers show evidence of platelet degranulation, thus activation can occur due to exposure of blood to roller pump segment and microbubbles may play an important role [5,7,10,39-42].

In our study, there was decrease in haemoglobin and haematocrit values in patients before haemodialysis session compared to controls. These values were increased in patients after haemodialysis. Renal system fails to sustain production of erythropoietin in CKD which leads to chronic anaemia [41]. Haemodialysis plays an important role in correction of anaemia that affects CKD patients which was proven in our study. There was no significant difference in platelet count in patients before and after haemodialysis. This finding was seen in study done by Bilgin A et al., also [43].

LIMITATION

The effect could have been studied better, if duration and frequencies of haemodialysis sessions, type of haemodialysis membrane and type of anticoagulants used were considered in the patient selection criteria.

The impact of insulin and oral hypoglycaemic agents and their duration on platelet indices and coagulation parameters were not considered in this study.

Long term complications could not be investigated since this study was for short duration.

CONCLUSION

Poor glycaemic control shows significant changes in MPV and PDW, thus platelet indices play a very crucial role in diabetic CKD patients. Hence, it aids as an important prognostic marker in assessing the complications. Basic coagulation profile like PT and APTT can predict long term complications of diabetic platelets in these patients. Haemodialysis preferred treatment of choice having more advantageous effect with few preventable causes which can be overcome with monitoring PDW and MPV values.

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PARTICULARS OF CONTRIBUTORS:

- Undergraduate Student, Department of Pathology, Sri Devaraj Urs Medical College, Kolar, Karnataka, India.
- Assistant Professor, Department of Pathology, Sri Devaraj Urs Medical College, Kolar, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Supreetha Megalamane,
Assistant Professor, Department of Pathology, Flat No 3, K Block New Doctors Staff Quarters. Sri Devaraj Urs Medical College,
Tamaka, Kolar-563101, Karnataka, India.
E-mail: drsupreethavv.2009@gmail.com

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