PLATELETS DYSFUNCTION IN PATIENTS OF END STAGE RENAL DISEASE

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ABSTRACT

Introduction: Patients with end – stage renal disease (ESRD) develop increased bleeding tendency, which is characterized by defective interaction of platelets with damaged sub endothelium due to impaired platelet functions. This study was carried out to demonstrate the aggregation defects in uraemic patients by using different platelet agonists.

Materials and Methods: A total of 57 subjects were included in the study. These were divided into two groups; 37 patients of ESRD on maintenance haemodialysis and 20 healthy adults as control. Complete blood count (CBC), urea and creatinine were carried out on all the samples. Aggregation studies were performed using chronology 490 – 2D Platelet Aggregometer. Adenosine diphosphate (ADP), collagen, ristocetin and arachidonic acid were used as agonists to perform aggregation studies and correlation of these parameters with Haemoglobin (Hb), Haematocrit (Hct), urea and creatinine were determined.

Results: All the subjects included in this study were evaluated for platelet aggregation in vitro. Percentages of maximal aggregation of platelets with ADP, collagen, ristocetin and arachidonic acid were significantly low in uraemic patients as compared to the control group. Aggregation with ristocetin was particularly reduced in uraemic patients (Mean 57.54 ± 23.85%) in comparison with controls (Mean 84 ± 6.01%). Inverse correlation of urea was found with ADP response (p value 0.028) and creatinine with arachidonic acid (p value 0.036) which was statistically significant. No correlation was found between haemoglobin, haematocrit and percentage of maximal aggregation after stimulation with collagen, ADP, ristocetin and arachidonic acid. Aggregation responses were reduced with almost all the agonists, especially ristocetin as compared to control samples.

Conclusion: This shows that defective platelet – vessel wall interactions play an important role in uraemic bleeding tendency.

Keywords: Platelet, ESRD, Haemodialysis.

INTRODUCTION

Uraemia patients are at an increased risk of bleeding.[1] The common complications are anaemia and haemorrhages which may also occur in end stage renal disease (ESRD) patients despite a normal coagulation profile[2]. This suggests that the bleeding tendency in these patients is due to platelet dysfunctions.[3,4] This dysfunction is multi-factorial in origin.[1,4] Level of von Willebrand factor is usually normal but with impaired function of GPIIb-IIIa receptors of platelets.[3] Various agonists like ADP, ristocetin, arachidonic acid and collagen can be used to stimulate platelets in order to measure their aggregation ability.[5,6] Objective of the present study was to demonstrate platelet functions in ESRD patients on haemodialysis.

SUBJECTS AND METHODS

The study was performed after approval by the Ethical Committee of the Hospital; informed consent were obtained from all the participants. Fifty seven subjects (20 healthy controls, and 37 patients of ESRD) were enrolled in the study. The diagnosis of renal disease was established by history, physical examination and laboratory investigations.

All of these patients were on haemodialysis therapy for more than 3 months. Patients were excluded from the study if they were found to have disseminated intravascular coagulation (DIC), congenital platelet disorders, liver disease, smoking, drugs affecting platelets within previous 14 days and any other disease affecting platelets. Control group included 20 healthy adults who did not have any history of bleeding and they did not take aspirin or any other anti-platelet drug for last 14 days. Descriptive information about the different study groups is provided in Table 1.

Blood Sampling
All the blood samples were collected by an expe-

rienced phlebotomist, immediately before the hae-modialysis session. Blood was collected blue top vacutainers (BD vacutainer sodium citrate 3.2%) in the ratio of 1 : 9 for aggregation studies, prothrombin time (PT) and activated partial thromboplastin time (APTT). Sample for complete blood count (CBC) was collected in BD vacutainer K_2 EDTA. For detection of urea and creatinine serum was separated, from gel separator tubes (BD vacutainer SSII) by centrifugation at 2400 × g for 10 minutes and was stored at - 40°C.

Platelet Aggregation
Platelet rich plasma (PRP) was prepared by centrifugation of citrated blood for 20 min at 100 × g. Platelet poor plasma (PPP) was obtained by centrifugation at 2400 × g for 20 min. These studies were carried out using Chronolog 490 – 2D Optical Platelet Aggregometer. Platelet count of platelet rich plasma (PRP) was adjusted to 250 x 10^9/L ± 50 × 10^9/L. Platelet aggregation response was tested with 2.5 µg/mL collagen (Chronolog Corporation), 4µM ADP (Chronolog Corporation), 0.5mM arachidonic acid (Chronolog Corporation) and 1 mg/mL ristocetin (Chronolog Corporation). Each of these reagents was added to 500µL of PRP (one agonist/cuvette) in the following amount. A volume of 1.25 µL of collagen, 2 µL of ADP, 5 µL of arachidonic acid and 4 µL of ristocetin. The minimum and maximum amplitudes of Chronolog 490 – 2D optical platelet aggregometer were adjusted with platelet rich plasma (0% transmission) and platelet poor plasma (100%

transmission), respectively. Tests were stopped after 6 minutes of stimulation and recorded as percentages of maximal aggregation with each agonist.

Statistical Analysis
The data was analysed using SPSS 16.0. Independent sample t test was applied to observe group differences. Pearson correlation was applied to observe correlations in quantitative variables. A *p-value* < 0.05 was considered statistically significant.

RESULTS
Number of subjects, sex and other laboratory characteristics of both study groups were expressed as mean ± standard deviation is provided in Table 1. Urea (128.38 ± 31.11 mg/dL) and creatinine levels (9.07 ± 2.84 mg/dL) in uraemic patients were quite high as compared to control group (29.15 ± 4.66 mg/dL and 0.81 ± 0.17 mg/dL respectively). Hematocrit levels were low in uremic patients (p < 0.05), with respect to the control group. No statistical difference was observed between the platelet counts in the uraemic (234.54 ± 99.961 × 10^9/L) and control group (267.6 ± 42.36 × 10^9/L).

Platelet Aggregation studies
Turbidimetric studies demonstrated consistent abnormalities in responses to aggregating agents in uraemic patients (Figure – 1). Function of platelets was affected with almost all the agonists tested (Table 2). However, these abnormal responses were most frequently observed with Ristocetin (with a percen-
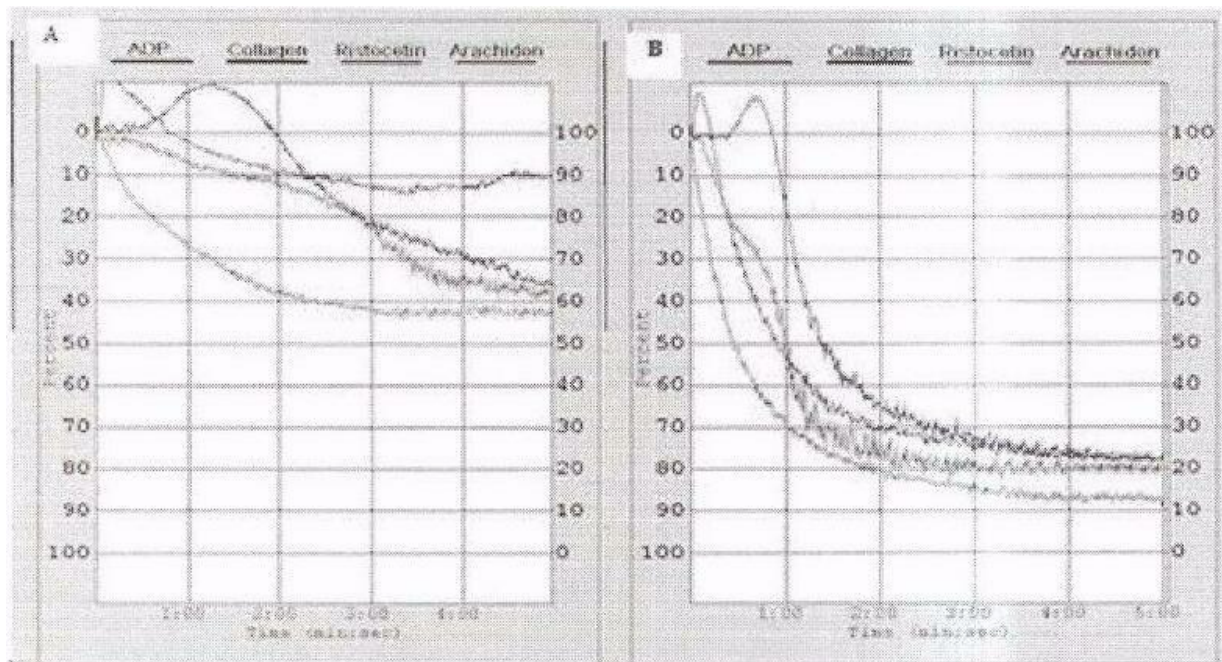

Fig. 1: *(A) Percentage of aggregation was low with all the agonists ADP, Collagen, Ristocetin and arachadonic acid. (B) Normal aggregation response to agonists obtained from an age matched control samples.*

tage of maximal aggregation 57.54 ± 23.85). Among the 37 uraemic patients, 26 demonstrated reduced platelet function in response to Ristocetin, 21 with collagen, 17 with ADP and 15 with arachidonic acid.

Correlation analysis
Inverse correlation of urea was found with ADP ($p < 0.05$, r = 0.13) and creatinine with arachidonic acid ($p < 0.05$, r = 0.119) which were statistically significant (Figure 2 & 3). However, no inverse relationship of urea and creatinine was found with ristocetin and collagen *(p > 0.05)* although the aggregation response was low.

DISCUSSION
Aggregation results in the present study showed reduced aggregation with almost all the agonists. Percentage of maximal aggregation with ristocetin was particularly reduced as compared to controls and was statistically significant *(p< 0.05)*. Similar findings have been reported by others.[7-9] Ristocetin response may be reduced due to GPIb or vWF deficiency. Hsu et al, found that GPIb mediated platelet aggregation in response to ristocetin was decreased even in the presence of increased von-Willebrand factor levels.[10] A similar study by Nomura et al, documented a reduction of GPIb expression by platelets of uraemic patients.[11]

Adenosine diphosphate stimulation involves release reaction and GPIIb – IIIa receptor activation by platelets. In our study platelets activation with ADP was significantly decreased *(p < 0.05)* as compared to controls. A similar study carried out by Salobir et al in Slovenia (2007) showed that platelets of uraemic patients had decreased aggregation in response to ADP. They also found a trend towards delayed platelet aggregation[12]. Comparable results have been reported in other studies.[7,13]
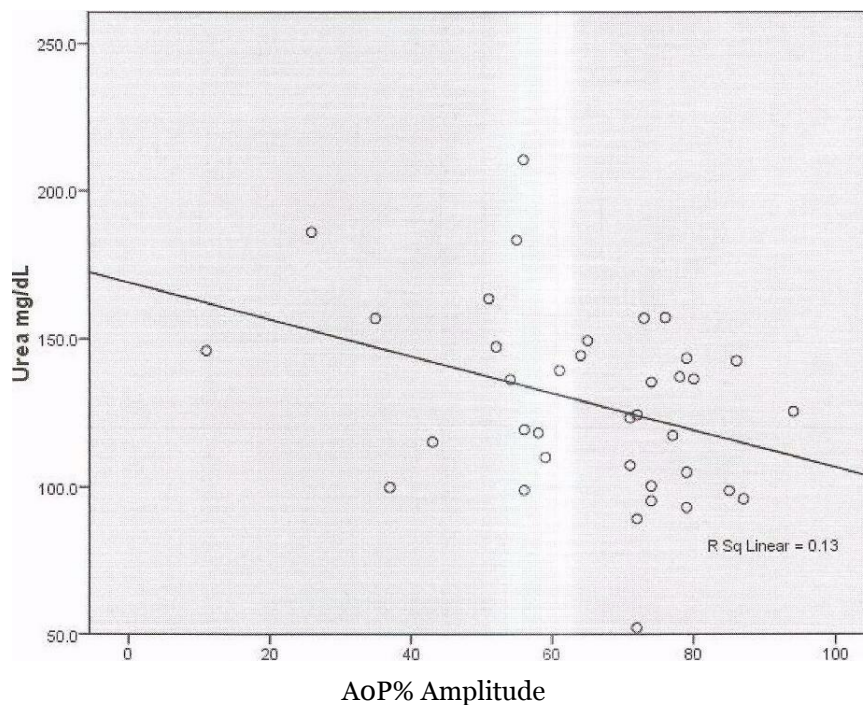

Fig. 2: *Correlation Curve of Urea and ADP among Uremic Patients.*
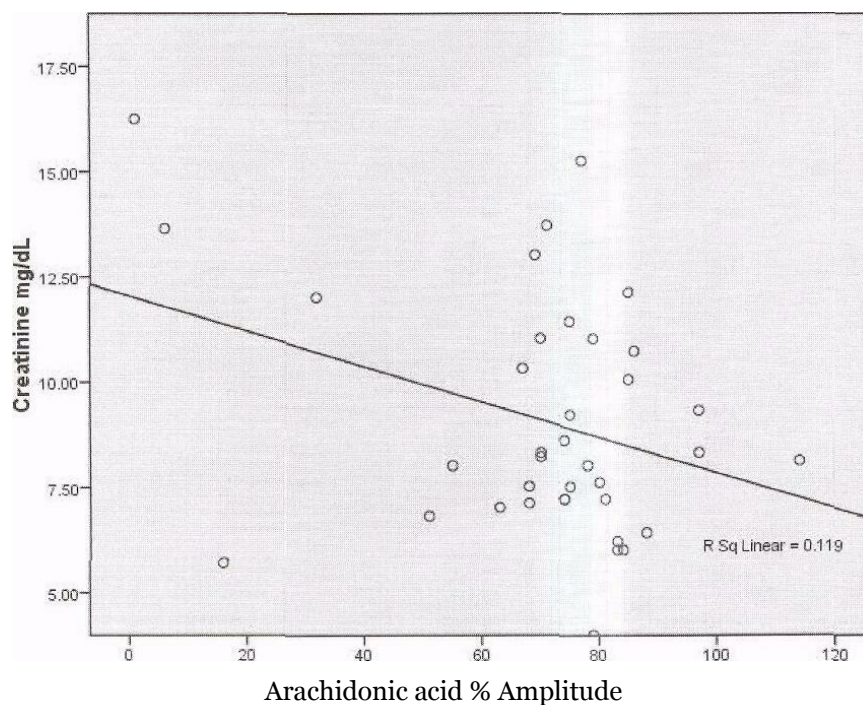

Fig. 3: *Correlation Curve of Creatimne and Arachidonic Acid in ESRD Patients.*

The present study showed decreased aggregation response to collagen in uraemic patients. Percentage of maximal aggregation was reduced as compared to controls in uraemic patients with a *p* value < 0.05. Comparable results were also found in

Stimulation of platelets with arachidonic acid was also reduced as compared to controls in our study and was statistically significant *(p < 0.05)*. Similar results were documented in other studies.[6,13] In contrast, Moal and others reported normal response to arachidonic acid.[9] This disparity of results can be due to differences in assessment methods or heterogenous subject populations.

An inverse correlation of urea only with ADP induced aggregation *(p < 0.05)* was found, whereas no correlation was observed with other agonists *(p > 0.05)*. This may be due to an acquired storage pool deficiency or GPIIb – IIIa deficiency produced by increased concentration of toxins including urea and nitric oxide donors such as guanidinosuccinic acid.[14-16] In our knowledge there is no such data available that reported correlation of urea with platelet agonists.

The present study reported a correlation between creatinine and arachidonic acid *(p < 0.05)*. No study was reported such a correlation. However, a study by Salvati and others reported a negative correlation between serum creatinine and the expression of glycoprotein GPIb by flowcytometry *(p < 0.0005)*.[17]

In our study platelet count was within the normal range or slightly low. Similar results were reported by others.[9,18] However, a number of studies reported low platelet count in uraemic patients.[19] This can be due to the activation of platelets due to interaction with dialysis membranes.[20] In this study samples were obtained before dialysis.

Anaemia is another frequent complication in uraemic patients.[21] All the patients were suffering from anaemia in our study. Previous studies also reported similar findings.[7,13,22] Red blood cells play an important role in facilitating the interaction of platelets with subendothelial surfaces[23]. Our study showed that there was no correlation among RBCs, haematocrit, haemoglobin and percentage of aggregation in response to different platelet agonists. However, a study conducted by Escolar and others stated that increase in haematocrit caused a significant increase in whole blood platelet aggregation in the uraemic patients under in vivo conditions provided by PFA 100.[13] On the other hand our study was in vitro on PRP and RBCs were mechanically removed. Therefore, the effect of RBCs and haematocrit on the platelet functions could not be tested. Since there is very limited data available on the effect of haematocrit on platelet aggregation, no in-vitro study on PRP was found. Further studies are required to check this effect using PFA – 100 which provides in vivo conditions for platelets.

An interesting finding in our study has been found that a few patients showed high responses to one or more agonists. These patients may be have smokers or they might be suffering from pseudo von-Willebrand disease.

ACKNOWLEDGEMENTS

Table 1: *Characteristics of the Controls and Patients.*

Parameter	Controls	Patients
Number of Subjects	20	37
Sex (male / female)	50 / 50	68 / 32
Age (years)	36.35 ± 14.86	40.57 ± 12.43
Urea (mg/dL)	29.15 ± 4.66	128.38 ± 31.11
Creatinine (mg/dL)	0.81 ± 0.17	9.07 ± 2.84
Hemoglobin (g/dL)	15.13 ± 0.85	9.13 ± 1.81
Hemocrit (%)	42.93 ± 1.26	26.19 ± 5.48
RBC Count (1×10^{12}L)	4.93 ± 0.28	26.19 ± 5.48
Platelet Count (1×10^9/L)	267.6 ± 42.36	234.54 ± 99.96
Prothrombin Time	13.40 ± 1.14	13.54 ± 1.26
Activated Partial Thromboplastin Time	37.60 ± 1.19	37.78 ± 1.29

Table 2: *Percentages of Maximal Aggregation of Patients and Controls.*

Agonists	Controls (Mean ± SD)	Patients (Mean ± SD)	p Value
Collagen (2.5 µg/mL)	78.01 ± 6.005	56.43 ± 24.58	0.000*
ADP (4 mM)	78.95 ± 5.04)	654.65 ± 17.85	0.001*
Arachidonic Acid (0.5 mM)	85.0 ± 6.53	70.27 ± 23.24	0.008*
Ristocetin (1 mg/mL)	84.0 ± 6.01	57.54 ± 23.85	0.00*

*Significant Value

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