INCREASED PLATELET AGGREGATION IN DIABETES MELLITUS

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SYNOPSIS

Platelet aggregation to adenosine diphosphate (ADP) was found to be increased in 48.2% of a group of 58 diabetic subjects. The platelet percentage aggregation (38.6 ± 11%) of those with clinical complications was significantly higher (p 0.001) than that of uncomplicated patients (21 ± 8%). The incidence of increased platelet aggregation in the female diabetics (11 out of 13) was significantly higher than that in the male cohort (17 out of 45) the reason for which is not known. Duration of disease, mode of therapy and isolated fasting blood glucose levels did not appear to influence platelet aggregability.

INTRODUCTION

Recent understanding of the pathogenesis of atherosclerosis suggests an important role for platelets (1). Endothelial injury results in platelet adhesion, platelet aggregation and the release of various intraplatelet substances including a mitogenic factor. This latter substance stimulates smooth muscle cells and fibroblasts of the vascular media to migrate into the intima and proliferate at the site of injury. Collagen and elastic fibres are laid down together with lipid deposition. Thus the groundwork for atherogenesis is initiated. The release reaction is a physiological function of the platelet. Its precise role in the development of macro-and microvascular complications in diabetes mellitus is not clear.

In the past decade platelet aggregometry has become an accepted indirect assessment of platelet function. Like other in vitro methods, a direct extrapolation to the physiological situation must be cautioned against. Despite its limitations, platelet aggregometry provides a simple and reproducible means of studying the platelet in disorders of hemostasis and more recently in arterial diseases. The method is based on the simple principle that when platelets aggregate in the path of a light beam, the optical density of the test system (the platelet-rich plasma) decreases. Changes in light transmission are recorded by a potentiometer connected to the aggregometer and they reflect the degree of platelet aggregation.

Results of platelet aggregation in diabetes mellitus have been conflicting. While some authors reported normal aggregation (2, 3) increasingly more are finding platelet hyperaggregation (4, 5, 6) which is believed to be caused by one or more plasma factors (7, 8, 9, 10). This study was undertaken to examine the behaviour of platelets in an unselected, heterogenous group of diabetic subjects. An attempt is made to correlate the results with fasting blood glucose levels, duration of disease, therapy and clinical complications.

MATERIALS AND METHODS

Fifty-eight diabetics from the Singapore Diabetic Society examined recently for clinical evidence of complications volunteered for this study. Controls (40 in total) consisted of regular blood donors, laboratory, nursing and medical staff from Medical Unit I, Singapore General Hospital. Subjects were instructed not to ingest aspirin or any other analgesics for at least 1 week prior to the study. After an overnight fast, venous blood samples were collected in siliconized tubes containing sodium citrate (1 ml 3.8% sodium citrate to 9 mls of whole blood). Platelet-rich plasma was collected by centrifugation at 1000 rpm for 10 minutes and platelet-poor plasma at 2,500 rpm for 20 minutes. A platelet count of the former was made using a Neubauer counting chamber to ensure a platelet concentration of 300,000/ul, adding platelet poor plasma if necessary to obtain the desired concentration. The platelet-rich plasma was tested within 2 hours of collection.

Platelet aggregation was evaluated turbidometrically in a Chronolog aggregometer (Model 440) coupled to a Rikadendi recorder (Model re 7811-1). ADP (Sigma Chemical Co, USA) was the principle agent used in the study. Platelet-rich plasma (0.45 ml) was pre-incubated at 37°C for 2 minutes, stirred magnetically at a speed of 1000 rpm. Platelet aggregation was induced by adding ADP (0.05 ml) to the platelet-rich plasma giving a final concentration of 2.5 uM. The result of the aggregation was based on the first phase of the aggregation curve and was expressed as the percentage change in optical transmission which will be referred to as the percentage aggregation (PA).

Fasting blood glucose levels and hemograms (hemoglobin, total leukocyte and differential counts, platelet count) were performed simultaneously on all subjects.

RESULTS

A total of 58 patients (mean age 49 years) and 40 controls (mean age 43 years) were studied. The normal platelet percentage aggregation (PA) based on the controls was 22 ± 6%. Twenty-eight out of the 58 diabetic patients had increased platelet aggregation (Group I) while 30 aggregated normally (Group II) (Table 1). The mean duration of disease was 12 years for Group I, and 11.5 years for Group II. The mean age for Groups I and II were 51 and 48 years respectively.

Eleven out of 13 female diabetic patients showed increased platelet aggregation to ADP compared with 17 out of 45 males (Table 2). Twenty-two patients in Group I had 1 or more complications (Table 3) whereas only 9 out of the 30 in Group II had evidence of vascular disease. Despite the high incidence of females with increased platelet aggregation, only 6 were observed to have complications. Of the remaining 5 it was noted that the duration of disease was less than 5 years. Sixteen out of the 17 male hyperaggregators had complications. The single male in Group I without complications has had diabetes for 3 years only (Table 4).

Group I had 7 patients with normal fasting blood glucose levels and 21 with levels above 100 mg%. In Group II, 10 had normal and 20 had elevated levels (Table 5). The mean fasting blood glucose levels for the 2 groups were not significantly different (Group I: 138 ± 9 mg%, Group II: 131 ± 8 mg%).

Eleven patients in Group I received insulin therapy compared to 5 in Group II (Table 6). Twelve received oral hypoglycemic agents in Group I whereas 22 were so treated in Group II. Of the 12 treated with oral agents in Group I, 8 were women (Table 7).

Hemograms were normal in all patients and controls.

Table 2
Diabetes Mellitus - Platelet aggregation and sex distribution

Patients	Male	Female
Group I	17	11
Group II	28	2
Total	45	13

Table 3
Diabetes Mellitus and Complications (Retinopathy, Neuropathy, Proteinuria, Hypertension, Ischemic Heart Disease, Peripheral Vascular Disease, Cerebrovascular Accident)

Patients	Complications	Multiple
Group I	22/28	14
Group II	9/30	6

DISCUSSION

Several observations can be made from this study. Firstly, there is a significantly higher incidence of female diabetics whose platelets hyperaggregated to ADP than males (85% versus 38%). The reason for this observation is not known. None of the women were on oral contraception or hormonal replacement. Aggregation studies were performed on each female subject during the first week of the menstrual cycle. Increased platelet aggregation in young, nondiabetic women in their early twenties had been reported (11). The mean age, however, of the 13 women diabetics in our study

Table 1
Mean duration of Diabetes Mellitus and Platelet Aggregation with ADP (PA = Percentage Aggregation)

Patients	PA	(Years) Mean Duration	(Years) Mean Age
(Group I) 28	>30%	12.0	51
(Group II) 30	21 ± 8%	11.5	48

Table 4
Diabetic Platelet Hyperaggregators and Complications

Sex	Complications	No Complications	Total
Male	16	1	17
Female	6	5	11
(Years) Duration of illness	12–15	3–5	12

Table 5
Platelet Aggregation and Fasting Blood Glucose Levels in Diabetes Mellitus

Patients	Fasting Blood Glucose 100mg%	Fasting Blood Glucose 100mg%
Group I	7	21
Group II	10	20

Table 6
Diabetes Mellitus and Treatment

Patients	Diet	Oral Agent	Oral Agent + Insulin	Insulin
Group I	2	12	3	11
Group II	3	22	0	5

Table 7
Group I Diabetic Patients and Oral Hypoglycemic Treatment

Group I	Total	Number Treated with Oral Agents
Female	11	8
Male	17	4
Total	28	12

Table 8
Patients on Oral Hypoglycemic Agents in Group I and Group II

Patients	Oral Agent	Percentage (%)
Group I	12/28	43
Group II	22/30	73

was 41 years.

Secondly, the results of isolated fasting blood glucose levels did not appear to affect platelet aggregability. Diabetic control, particular longterm control, is more accurately reflected by the levels of glycosylated hemoglobin than by isolated blood glucose levels. Our ongoing study on platelet aggregation with respect to glycosylated hemoglobin levels will, we hope, answer the question as to whether diabetic control influences platelet aggregability.

Thirdly, on first glance, Group II had a proportionately larger number of patients on oral hypoglycemic agents (73%) compared to Group II (43%) (Table 8). Sulfonylureas such as gliclazide and glyburide had been shown to inhibit platelet aggregation independent of their glucose-lowering effect (12). Evidence for tolbutamide or chlorpropamide influencing platelet function is inconclusive. Our patients were treated with tolbutamide or chlorpropamide. None of them received gliclazide or glyburide. It is of further interest to note that 8 out of the 11 females in Group I received oral hypoglycemic therapy. This would suggest that the oral agents used could not have exerted a significant inhibitory effect on their platelet aggregation.

Fourthly and finally our study demonstrated a positive association between increased platelet aggregation to ADP and the presence of clinical complications. This was particularly striking in the male diabetics where 16 out of 17 patients in Group I had 1 or more complications. In both sexes the uncomplicated hyperaggregators had disease duration of less than 5 years. It would be interesting to follow this subgroup to see when complications appear and whether all develop complications.

Like other workers in this field we are faced with the issue of whether altered in vitro platelet behaviour has any bearing on physiological events in the diabetic patient. In vivo studies in diabetics showing increased platelet prostaglandin synthesis (13, 14, 15), increased plasma beta-thromboglobulin concentration (16, 17), the presence of circulating platelet aggregates in diabetics with microvascular disease (17) and increased platelet turnover (18, 19) suggest that platelet hyperaggregation in vitro may relate to events in the body. Whether increased platelet aggregation is the cause or consequence of diabetic vascular disease, the result of an intrinsic platelet dysfunction or the result of a plasma factor are all unsettled issues. The link between platelets and diabetic vascular disease is still circumstantial. An indirect approach to solving this puzzle lies in the prevention of vascular complications in the diabetic

through the longterm administration of antiplatelet drugs. Two prospective studies are currently underway in the United States (10). The Veterans Administration is looking at the effect of aspirin and dipyridamole on diabetic lower extremity vascular disease while the National Institute of Health is studying the effect of antiplatelet agents on background diabetic retinopathy. Decisions concerning the use of such agents in clinical practice in the diabetic patient will likely be dictated by the results of these studies.

CONCLUSION

Approximately 50% of the diabetic patients in the study showed increased platelet aggregation to ADP. A positive association exists between increased platelet aggregation and the presence of clinical complications particularly in the males where 16 out of 17 hyperaggregators had 1 or more complications. Duration of disease, mode of therapy and isolated fasting blood glucose level had no bearing on platelet aggregability.

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