Original Article

Platelet Hyper-Aggregation and Abnormalities of Coagulation Factors in Young Diabetic Patients

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Abstract

Diabetic vascular disease is conveniently divided into two main categories: microvascular diseases and macrovascular diseases. The changes involving the smallest blood vessels - the capillary and pre-capillary arterioles- are microvascular changes. The cross sectional case-control study was done on forty young diabetic patients for their platelet aggregation response to ADP, plasma fibrinogen and vWF in relation to the age and BMI matched 10 nondiabetic control subjects (Age in years : 23.7 ± 2.26 in control vs 24.33 ± 3.29 in subjects, and BMI in kg/m2. The lipid profile, C-peptide, C-peptide: glucose and other anthropometric measurements of the diabetic and control subjects were also measured to find out any possible correlation. The platelet aggregation with ADP in diabetics was found to be significantly higher (in diabetics 76.56 ± 16.92 percent compared to controls 62.90 ± 12.35 percents; (P< 0.27). Plasma fibrinogen and plasma von Willebrand factors were found to be significantly higher in young diabetics compared to the non-diabetic counterparts (Plasma fibrinogen in mg/l; 1075.90 ± 455.16 in control vs 1569.15 ± 731.42 in diabetic, P<0.048; plasma vWF in iu/ml; 1.372 ± 0.340 in control vs 1.884 ± 0.51 in diabetic, P<0.001). Fasting plasma glucose was higher and C-peptide - glucose ratio lower in diabetic subjects compared to the controls (Fasting plasma glucose in mmol/l; 3.48 ± 0.38 in control vs 16.02 ± 8.58 in diabetic; C-peptide-glucose ratio 0.426 ± 0.133 in controls vs 0.116 ± 0.105 in diabetics, P<.001). Thus hyperglycemia, endothelial dysfunction, elevated vWF and insulin resistance were supposed to be interlinked.

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Introduction

Vascular complications are common in diabetes mellitus and are leading causes of morbidity and mortality due to the disease. Platelet hyperaggregation and coagulation factor abnormalities have been implicated in the increased prevalence of vascular complications. However, their precise role is uncertain and it is also not known whether they are primary or secondary in nature. Moreover, the precise role of hyperglycemia in the development of these abnormalities have remained similarly uncertain. To help in understanding the pathogenesis of platelet hyperaggregation and coagulation factor abnormalities in diabetes mellitus and to explore the role of hyperglycemia, this study was done on a group of young, non-obese, normotensive and untreated Bangladeshi diabetic subjects. This has enabled to explore the role of hyperglycemia in the absence of the confounding variables and also to investigate the interrelationship among the presumed risk factors leading to vasculopathy.

Diabetic vascular disease is conveniently divided into two main categories: microvascular diseases and macrovascular diseases. The changes involving the smallest blood vessels - the capillary and pre-capillary arterioles- are microvascular changes. Sequential features of these changes are: (a) pre-capillary vasodilatation and hyperfiltration of fluid, (b) increased basement membrane formation and (c) breakdown of normal chargebarrier causing increased transcapillary escape of albumin. In advanced cases, loss of vessel caliber and vascular occlusion ensues[1].

Microvascular changes involve the retina, kidneys, vasa nervosum and heart. Pathologically,microvascular involvement of glomeruli leads to diabetic nephropathy and retinal involvement gives retinopathy. Small vessel diseases also involve the heart and cardiomegali with heart failure has been described with patent coronary arteries. It has long been postulated that microvascular lesions play a role in the production of macrovascular diseases also. But it needs further study to confirm the speculation[2].

The hall mark of macrovascular complications is atherosclerosis both in nondiabetics and diabetics with he same sequence of events of pathogenesis as (a) endothelial injury; (b) platelet adhesion and aggregation with the release of mediators (c) smooth muscle proliferation and migration into sub-endothelium at the area of damage; (d) accumulation of extracellular matrix, lipids and lipoproteins; and (e) thrombosis, the process is reversible with counter-regulatory events and accelerated by impaired host response to any of the above events[3].

But in diabetics these events appear earlier and are more extensive and associated with higher mortality and morbidity.

A reason for increased platelet aggregability in platelet rich plasma from diabetic subjects is platelet-plasma interaction. A-"platelet aggregation enhancing factor" has been described to be present in many diabetic patients. This plasma factor would interact would interact with platelets to make them more sensitive to ADP- induced aggregation. Plasma factor activity was greatest in patients with retinopathy and nephropathy[4]. However the source pf PAEF activity has not been fully identified. Some of the logical candidates would include vWF, fibrinogen, immune complexes, free fatty acids, other lipids and lipoproteins. Again there are controversies about the role of fibrinogen and vWF (coagulation factors) in inducing vascular complications in diabetics. Some studies show convincing evidences and some are inconclusive or against the proportion. It is not clear whether the prognostic value of vWF is related to it's specific functions of enhancement of platelet adhesion and factor VIII availability, or whether it is simply an indication of endothelial injury and dysfunction. But this endothelial dysfunction and injury may be responsible for increased risk of nephropathy and cardiovascular disease. Elevated vWF may also precede initial neuropathy-both autonomic and peripheral. vWF factor may also predict alterations in nerve function independent of glycemic control. But retinopathy was not found to be related with alteration in vWF level in type-1 diabetic subjects[5].

Materials and Methods

The cross sectional analytical study was done on 50 patients in Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic disorders (BIRDEM). Forty (40) subjects were newly diagnosed young (age range 24±3, 18 male 22 female) diabetes, consecutively attending the Out-patients Department of BIRDEM, Dhaka.

Control subjects were selected from friends of the patients without any family history of diabetes up to second generation (n =10) within 5 years age band. Control subjects were matched for BMI, urban/rural setting and socio-economic status with their diabetic counterparts.

Exclusion criteria

1. Diabetic subjects suffering from chronic illness, nondiabetic renal disease and pregnancy were excluded from the study.

2. Diabetic subjects with ketosis and history of antidiabetic treatment were also excluded.

3. Both the diabetic subjects and healthy controls with parental history of essential hypertension were also excluded.

Detailed sociodemographic data, Anthropometric measurements, family history of diabetes, medical history, physical and clinical examinations were done on the very first day of visit.Platelet aggregation was measured by Optical Aggregation Test and plasma Fibrinogen and von WilleBrand Factor (vWF : Ag) was measured by radio-immuno-diffusion method.

Results

Fifty subjects were studied in this project. Forty were young diabetic subjects (under 30 years of age) and ten were control. The mean (± SD) age of the diabetic subjects was 24.33 ± 3.29 years and in control subjects the mean age was 23.70 ± 2.26 years. There was no significant difference in age between control and the diabetics.

The BMI of the two groups was also matched. (BMI in kg/m2 20.68 ± 5.78 in diabetics and 20.52 ± 2.05 in controls).

The groups had similar systolic blood pressure (blood pressure in mm. of Hg: 107.5 ± 8.58) in controls and 108.13 ± 13.7 in diabetics) but significant difference was observed in diastolic blood pressure (blood pressure in mm of Hg : 63.50 ± 4.74 in control vs 71.38 ± 9.67 in diabetic; P<0.001) (Table-I).

Their mid-arm circumference and sub-scapular-triceps ratio were also matched.

Results are expressed as mean ± SD. Unpaired student's t-test was performed.

Table - I: Anthropometric features of different groups of study subjects.

Groups	Age (years)	BMI (kg/m²)	MAC (cm)	STR	WHR	sBP (mmHg)	dBP (mmHg)
Control (n= 10)	23.70±2.26	20.52±2.05	22.80±2.40	1.46±0.59	0.81±5.97	107.50±8.58	63.50±4.74
Diabetic (n=40)	24.33±3.29	20.68±5.78	22.81±4.90	1.80±0.48	0.88±7.50	108.13±13.7	71.38±9.67

N = number of subjects, BMI = Body mass index, MAC = Mid arm circumference, STR = Subscapular triceps ratio

WHR = Waist hip ratio, sBP = Systolic blood pressure, dBP = Diastolic blood pressure

Both fasting plasma glucose and C-peptide : glucose ratio were highly significant in diabetic subjects sompaired to the controls.

Fasting plasma glucose in mmol/l; 3.48 ± 0.38 in control vs 16.02 ± 8.58, P<0.00. C-peptide : glucose ratio in controls 0.426 ± 0.133, vs 0.116 ± 0.105, in young diabetics. But no significant difference was found in C-peptide levels in control and diabetic groups (Table-II).

Results are expressed as mean ± SD. Unpaired student's t-test was performed.

Table- II: Glycemic status of different groups of study subjects.

Groups	F-glucose (mmol/l)	C-peptide (ng/ml)	C-peptide : Glucose
Control (n= 10)	3.48 ± 0.38	1.44 ± 0.33	0.426 ± 0.133
Young diabetics (n=40)	16.02 ± 8.58	130. ± 0.84	0.116 ± 0.105
t/p value	9.21/0.00	0.53/0.59	6.83/0.00

N = number of subjects, F = Glucose; fasting glucose, C-peptide = Connecting peptide

Platelet aggregation by ADP in controls was found to be 62.90 ± 12.35 percent compared to the diabetics 76.56 ± 16.92 percent. Data indicated that there was increase in percent of aggregation induced by ADP in diabetics and found to be significant (P< 0.027) (Table-III).

But no difference was observed in platelet count between two groups.

Table- III: Platelet count, platelet aggregation, plasma fibrinogen and von Willebrand factor status of the study subjects.

Groups	Platelet count /mm³	Platelet aggregation by ADP %	Plasma fibrinogen mg/l	Plasma vWF iu/ml
Control (n= 10)	207300 ± 44236	62.90 ± 12.35	1075.90 ± 455.16	1.372 ± 0.340
Diabetic (n=40)	221650 ± 61555	76.56 ± 16.92	1569.15 ± 731.42	1.884 ± 0.51
t/p value	0.691/0.493	2.283/0.027	2.027/0.048	3.818 / 0.001

Plasma fibrinogen and plasma von Willebrand factors were found to be significant in young diabetics compared to the non-diabetic counterparts. Specially plasma vWF was found to be highly significant.

Plasma fibrinogen in mg/l; 1075.90 ± 455.16 in control vs 1569.15 ± 731.42 in diabetic, P< 0.048). Plasma vWF in iu/ml; 1.372 ± 0.340 in control vs 1.884 ± 0.51 in diabetic, P <0.001 (Table-IV).

Table-IV: Pearson correlation coefficient between von Willebrand factor and anthropometric

Groups	BMI		WHR		STR		MAC	
	r	p	r	p	r	p	r	p
Diabetic (n=40)	0.006	0.970	-0.058	0.722	0.037	0.821	0.066	0.684

BMI = Body mass index, WHR = Waist hip ratio, STR = Subscapular triceps ratio, MAC = Mid arm circumference

r = Pearson correlation coefficient, P = Significant value for 'r', N = number of subjects

Table- V: Pearson correlation coefficient between plasma fibrinogen and anthropometric status

Groups	BMI		WHR		STR		MAC	
	r	p	r	p	r	p	r	p
Diabetic (n=40)	0.295	.065	-0.105	0.518	0.006	0.971	0.237	0.140

Statistical analysis were performed by Bivarient correlation of pearson coefficient

Discussion

There is now overhelming evidence that a procoagulant state exists in diabetic subjects, but there is a great deal of controversy regarding the primary or secondary nature of this state. The question is important to be solved because it addresses the basic question about the causal relation of coagulation factors in the genesis of macro and microvascular diseases in diabetes mellitus. In turn, this have important implication in the management of diabetes mellitus and its complications.

The role of platelets in arterial in arterial thrombosis is similar to their role in hemostasis. In the hemostatic. Reaction, platelets adhere to the damaged vessel wall and aggregate to each other, releasing ADP and initiating the chain of events leading to the formation of a white thrombus[6].

Substantial endothelial damage and contact of platelets with collagen fibers, basement membrane and microfibrils of the vessel wall are required to initiate arterial thrombosis.There is a postulation that platelet activation may lead to the release of mediators which may secondarily influence the development of coagulation factor abnormalities[7].

The present findings do not seem to support the view since there was no correlation between platelet aggregation and any of the coagulation factors. Also, no correlation was observed in between any of the two coagulation factors suggesting that the change in the levels of these factors may reflect primary changes which are still not clearly understood. A genetic predisposition which may coexist with the diabetic condition may be a possibility. However, further detail studies on the metabolic changes in relation to endothelial function, may generate valuable results for solving this issue.

Endothelial damage or dysfunction causing angiopathy is the main cause of morbidity and mortality in diabetes mellitus. Glycemic control seems to be insufficient to normalize vWF but other specific treatments required. vWF appears to be a predictive marker of diabetic nephropathy and neuropathy which suggests that endothelial dysfunction precede the onset of diabetic macroangiopathy.At present, it is not known, whether high vWF levels are inherent to this physiopathology of diabetes, nor whether diabetes induces endothelial dysfunction through other ways[8].

Hyper fibrinogenemia has emerged as an independent predictive risk factor for CHD. Indeed hyperfibrinogenemia now appears to challenge the rank power of hypercholesterolemia as a predictive and independent risk factor. These varied observations provide an important link between altered haemostasis, thrombosis and clinical CHD. Intervention strategies are targeted to the areas of thromboresistance also[9].

From the present study, it was observed that in young diabetics, platelet hyperaggregation and coagulation factor abnormalities appear quite early and seem to play important role in the natural history of vasculopathy in diabetes mellitus. Diabetic condition (as evidenced by hyperglycemia), by itself, may create platelet hyperaggregation and coagulation factor abnormalities. However, there seems to be no straightforward relationship between those abnormalities and the degree of hyperglycemia. Factors other than glucose may be involved in the genesis of the above abnormalities as primary or secondary events. Platelet hyperaggregation, hyperfibrinogenemia and increased vWF do not influence each other in a direct manner.

More detailed studies on genetic factors and endothelial metabolic abnormalities, with long-term follow-up, may clarify the primary or secondary nature of procoagulant abnormalities in diabetes mellitus.

References

1. Sasaki A, Uchara M, Horsuchi M, Hasagawa K. A long term follow up study of Japanese diabetic patients mortality and cause of death. Diabetologia 1983;25:309-312.

2. OMP. Ganda. Pathogenesis of microvascular disease. Diabetes Nov 1979;19: 37.

3. John A. Colwell, Maria Lopes Virella, Perry V. Halushka. Pathogenesis of atherosclerosis in DM. Diabetes Care Jan-Feb, 1981;4:122.

4. John A. Colwell, Maria Lopes Virella, Perry V. Halushka. Review of the development of large-vessel disease in Diabetes Mellitus. Am. J. Med Nov 28, 1988;84:114-115.

5. Stehouwer CD. Endothelial dysfunction preceeds development of microalbuminuria. Diabetes 1995;44:330.

6. John A. Colwell. Maria Platelet function & platelet-plasma interactin. IN: Harold Rifkin & Daniel Porte, Jr. (editors). "Diabetes Mellitus- Theory and Practice. Ed-4, London: Elsevier; 1990;0253.

7. Tscheepe D, Rauch U, Schwippert B. Platelet-Leukocyte cross-talk in diabetes mellitus. Horm. Metab Res 1997;29:631-635.

8. L. Kessler, (1) M.I. Wiesel (2), J.M. Mossard (3), J.P. Cazenave (2), M. Pinget (1) "vWF in diabetic angiopathy". Diab-Metab 1998;24:327-36.

9. Stahl SM. "Platelets as pharmacologic models for the receptors and bio-chemistry of monoaminergic neurous. In: G Richard Lee, Thomas C. Bithel, John Foerster (editors). Wintrob's Clinical Hematology. 9th Edition. London. Lea & Febriger, 1993;514.