ORIGINAL PROF-591

PLATELET AGGREGATION STUDIES IN DIABETIC RETINOPATHY

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ABSTRACT

Platelets have been implicated in the genesis of vascular complications of diabetes mellitus. This is shown by platelet hyperaggregability in diabetic subjects. We studied the platelet aggregation pattern in 200 diabetic subjects. 100 with diabetic retinopathy and 100 without retinopathy. Platelet aggregation was found to be enhanced in all 200 subjects studied. ADP, epinephrine, collagen and arachidonic acid were used as agonists to induce platelet aggregation. The results were compared with 50 healthy age, sex matched non diabetic controls. We did not find any significant difference between the platelet aggregation of diabetic patients with and without diabetic retinopathy.

INTRODUCTION

Diabetes Mellitus (DM) is characterized by sustained hyperglycemia secondary to lack or diminished efficiency of endogenous insulin. Type I, insulin dependent diabetes (IDDM) develops most frequently between 10-20 years of age. While type II non insulin dependent diabetes (NIDDM) develops between the ages of 50-70 years[1]

The complications associated with DM were first recognized by Blumenthal et al. 1962[2]. Vascular disease is the largest and most interactable problem in clinical diabetes. It involves arterioles and capillaries[3]. Diabetic micro-angiopathy involves small vessels of kidney, nervous system and retina[4]. Diabetic retinopathy (DR) is one of the serious manifestations of the micro-angiopathy. Various studies report a prevalence of about 52% amongst diabetic population[5,6]. This is higher in IDDM than in NIDDM and DR is the most common cause of blindness in individuals between the age of 20-65 years. There is an overwhelming evidence that platelets from diabetic individuals are hyper reactive, not only when microvascular complications are apparent, but already at an early stage of the disease[7].

Clinically, the three main types of DR are background, pre-proliferative and proliferative. The pathogenesis of microvascular occlusion involves thickening of the basement membrane, endothelial cell damage and proliferation in the capillaries along with deformation of red blood cells resulting in decreased oxygen transport and increased stickiness and aggregation of platelets[8,9]. It is generally accepted that growth factors play an important role in DR. Elevated concentration of platelets derived growth factor (PDF) in the vitreous of the patients of DR have been observed. It is thought that ischemia is a result of microvascular occlusion which might be a strong stimulator of growth factor production. Neovascularization is thought to be caused by growth factors released from the hyper aggregable platelets in an attempt to revascularize hypoxic areas of retina[10,11].

Levels of PDGF were significantly higher in DR patients than the non DM group ($P<0.01$)[12]. There is decreased production of prostacycline which is a potent inhibitor of platelet aggregation[13,14]. Plasminogen activators have been reported to be low in diabetics which play an important role in dissolution of thrombin[15]. These functional changes in the hemostatic

system do not relate to the age and type of DM[16].

SUBJECTS & METHODS

The present study was conducted on a total number of 250 subjects out of which 200 were diabetic and 50 age and sex matched controls. Detailed clinical examination including fundoscopy was carried out. Patients with known H/O ischemic heart disease or hypertension were not included in the study (table I).

Table-I. Age and sex distribution of diabetic patients and normal controls.

Group	Sex	No of Patients	Age (years)	
			Range	Mean ±SD
Control	Male	25	14-70	46.28 ±16.98
	Female	25	16-70	46.44 ±16.91
IDDM	Male	10	14-30	21.70 ±4.36
	Female	10	16-30	21.20 ±3.76
IDDM (Comp)	Male	10	18-43	31.00 ±7.06
	Female	10	24-41	33.20 ±5.49
NIDDM	Male	40	36-65	48.60 ±6.56
	Female	40	30-65	47.30 ±8.05
NIDDM (Comp)	Male	40	48-72	57.30 ±6.56
	Female	40	45-70	55.90 ±6.15

Group A included 100 diabetics with ophthalmoscopic evidence of retinopathy. This group comprised of 40 NIDDM and 10 IDDM females, 40 NIDDM and 10 IDDM males. Group B included 100 diabetics with no clinical evidence of retinopathy. The distribution of the subjects was the same as that of group A. No patient had taken any drug known to effect platelet function for one week prior to the test.

10 ml of fasting blood was drawn aseptically in a plastic tube containing 9 ml of 3.8% trisodium citrate and was gently mixed. The sample was centrifuged at 1000 RPM for 10 minutes to get platelet rich plasma (PRP). While the platelet poor plasm (PPP) was obtained by centrifugation at 3000 RPM for 20 minutes. Platelet count was adjusted between 200-400-10-9/1 by diluting with PPP. Platelet aggregation (PA) was performed within the 6 hours. Of the sample collection on Chronolog Aggregometer Model No. 430. The agonists used were as follows;

1. ADP (1.0, 2.5, 5.0 and 10.0 umol).
2. Epinephrine (1.0, 2.0 and 20.0 umol)
3. Collagen (1.0, 2.0 and 4.0 ug/ml).
4. Arachidonic acid (125, 250 and 500 ug/dl

The following parameters were included in PA studies.

1 Intensity of aggregation (%, IR)
2 Rate of aggregation (%/m, RA)
3 Lag Phase (sec. LP)
4 Spontaneous aggregation (SA).

The statistical analysis was done by applying student "t" test.

RESULTS

Intensity and Rate of Aggregation

Group A: IA and RA

IA and RA were significantly higher in patients of NIDDM and IDDM with DR as compared to controls with all the concentrations of ADP, epinephrine, collagen and archidonic acid ($P<0.001$) (Table II to IX).

Group B: IA and RA.

IA and RA were significantly higher in patients without vasculopathy as compared to controls with all the concentrations of the agonists used (P<0.001) (Table II to IX).

No significant differences were however, noted between diabetics with or without DR.

Table-II. Intensity of aggregation and rate of aggregation with different concentrations of ADP in diabetic female patients and normal controls.

Group	Intensity of aggregation (%)				Rate of aggregation			
	1.0 umol	2.5 umol	5.0 umol	10.0 umol	1.0 umol	2.5 umol	5.0 umol	10.0 umol
Control	-	39.4 ±11.3	54.4 ±6.5	66.12 ±6.7	-	1.35 ±0.4	1.61 ±0.42	2.24 ±0.4
IDDM	50.7 ±4.0	64.2 ±3.65	72.2 ±2.8	80.2 ±4.4	1.87 ±0.2	2.33 ±0.4	2.9 ±0.73	3.56 ±0.5
IDDM (Comp)	54.3 ±3.6	65.5 ±2.73	75.7 ±2.8	84.9 ±3.3	1.63 ±0.1	2.46 ±0.2	3.05 ±0.1	3.98 ±0.1
NIDDM	40.7 ±24	59 ±13.7	68 ±7.7	76.58 ±7.1	1.39 ±0.8	2.08 ±0.6	2.59 ±0.7	3.39 ±0.9
NIDDM (Comp)	53.8 ±13	61.67 ±11.4	68.78 ±5.9	75.28 ±6.2	1.57 ±0.4	2.04 ±0.5	2.45 ±0.49	2.8 ±0.5

Table-III. Intensity of aggregation and rate of aggregation with different concentrations of ADP in diabetic male patients and normal controls

Group	Intensity of aggregation (%)				Rate of aggregation			
	1.0 umol	2.5 umol	5.0 umol	10.0 umol	1.0 umol	2.5 umol	5.0 umol	10.0 umol
Control	-	44.76±6.97	56.36±6.97	67.72±4.82	-	1.13±0.26	1.54±0.35	1.99±0.4
IDDM	52.1 ±3.48	65.1±3.58	72.9±2.17	81.3±3.66	2.01±0.25	2.4±0.43	3.11±0.63	4.08±0.58
IDDM (Comp)	55.5 ±6.09	676±3.58	76.03±4.73	83.9±4.16	1.76±0.24	2.24±0.29	2.94±0.29	3.91±0.46
NIDDM	42.35±23.2	60.6±13.3	68.88±7.1	77.05±7.75	1.4±0.83	2.13±0.75	2.64±0.81	3.45±1.08
NIDDM (Comp)	60.8 ±5.66	68.35±6.09	74.17±7.16	80.97±8.41	1.94±0.5	2.49±0.64	3.04±0.85	3.5±1.09

Table-IV. Intensity of aggregation and rate of aggregation with different concentrations of EPI in diabetic female patients and normal controls

Group	Intensity of aggregation (%)			Rate of aggregation		
	1.0 umol	2.0 umol	20.0 umol	1.0 umol	2.0 umol	20.0 umol
Control	-	40.76±13.05	61.68±8.52	-	1.11±0.47	1.50±0.52

Table-IV. Intensity of aggregation and rate of aggregation with different concentrations of EPI in diabetic female patients and normal controls

Group	Intensity of aggregation (%)			Rate of aggregation		
IDDM	48.6±4.67	62.7±4.98	75.3±3.58	1.61±0.19	1.93±0.25	2.37±0.39
IDDM (Comp)	48.8±2.68	64.0±5.25	80.7±2.15	1.56±0.13	1.97±0.13	3.19±1.6
NIDDM	39.4±23.66	59.38±14.09	74.2±2.15	1.2±0.73	1.74±0.68	2.56±0.82
NIDDM (Comp)	52.2 ±15.83	62.6±11.39	74.3±6.18	1.62±0.54	2.15±0.47	2.62±0.48

Table-V. Intensity of aggregation and rate of aggregation with different concentrations of EPI in diabetic male patients and normal controls

Group	Intensity of aggregation (%)			Rate of aggregation		
	1.0 umol	2.0 umol	20.0 umol	1.0 umol	2.0 umol	20.0 umol
Control	-	43.84±9.9	52.44±9.26	-	0.9±0.12	1.22±0.34
IDDM	48.8±3.1	64.7±3.77	79.3±1.27	1.71±0.18	2.05±0.27	2.81±0.33
IDDM (Comp)	54.25±5.58	64.6±5.14	80.4±4.5	1.64±0.3	2.04±0.42	2.94±0.45
NIDDM	40.73±23.4	60.02±13.94	74.45±8.76	1.24±0.74	1.88±0.72	2.64±0.83
NIDDM (Comp)	61.0 ±6.04	68.97±4.99	78.35±8.39	2.04±0.47	2.78±0.67	3.18±0.9

Table-VI. Intensity of aggregation, rate of aggregation and lag phase with different concentrations of collagen in-diabetic female patients.

Group	Intensity of aggregation (%)			Rate of aggregation			Lag phase (Sec)		
	1.0 ugm/mol	2.0 ugm/mol	4.0 gm/mol	1.0 ugm/mol	2.0 ugm/mol	4.0 ugm/mol	1.0 ugm/mol	2.0 ugm/mol	4.0 ugm/mol
Control	-	53.1±8.6	65.6±7.3	-	1.5±0.4	2.0±0.5	-	47.9±11	27.1±9
IDDM	70.0±2.5	74±2.5	81.8±3.9	2.3±0.2	2.8±0.2	3.5±0.3	30±6.8	14.9±0.6	7.6±5.2
IDDM (Comp)	69.5±1.9	80.6±2.0	87.1±2.4	2.0±0.2	3.3±0.2	4.0±0.1	21±5.4	12.2±5.2	7.4±3.8
NIDDM	54.4±22	68.4±8.4	77.7±8	1.7±0.9	2.7±0.8	3.6±1.0	32.9±17	26.9±15	14.4±11
NIDDM (Comp)	57.6±17.7	70.1±9.4	76.1±6.6	1.8±0.6	2.6±0.4	3.1±0.6	20.6±12	12.4±9.8	7.4±5.9

Table-VII. Intensity of aggregation, rate of aggregation and lag phase with different concentrations of collagen in diabetic female patients and normal control.

Group	Intensity of aggregation (%)			Rate of aggregation			Lag phase (Sec)		
	1.0 ugm/ml	2.0 ugm/ml	4.0 ugm/ml	1.0 ugm/ml	2.0 ugm/ml	4.0 ugm/ml	1.0 ugm/ml	2.0 ugm/ml	4.0 ugm/ml
Control	-	55.16±8.9	68.4±5.2	-	1.3±0.4	1.8±0.5	-	53.8±9.1	35.2±10
IDDM	67.5±4.05	73.8±3.6	81.8±3.3	2.1±0.2	2.7±0.4	3.9±0.6	29.0±6.5	14.8±4.7	8.1±5.1
IDDM (Comp)	65.3±3.07	76.2±3.1	83.5±3.2	2.2±0.4	3.0±0.4	3.8±0.5	21.0±5.4	12.2±5.2	7.0±1.5
NIDDM	52.1±24	68.4±7.7	78.1±7.1	1.7±0.9	2.6±0.7	3.5±1.0	31.4±18.7	25.8±16	14.7±12
NIDDM (Comp)	64.8±6.55	73.5±5.1	80.7±7.8	2.2±0.5	2.9±0.7	3.5±1.0	20.6±12.3	12.4±9.8	8.04±6.0

Table-VIII. Intensity of aggregation, rate of aggregation and lag phase with different concentrations of A.A. in diabetic male patients and normal.

Group	Intensity of aggregation (%)			Rate of aggregation			Lag phase		
	125 ugm/ml	250 ugm/ml	500 gm/ml	125 ugm/ml	250 ugm/ml	500 ugm/ml	125 ugm/ml	250 ugm/ml	500 ugm/ml
Control	-	52.4±11	67.8±5.5	-	1.4±0.5	1.9±0.5	-	42.7±12.1	11.6±3.0
IDDM	64.7±4	72.8±2.2	81.7±2.1	1.9±0.22	2.8±0.4	3.8±0.43	30±3.9	13.3±2.9	2.9±2.6
IDDM (Comp)	60.4±3	72.3±2.9	82.9±3.3	1.8±0.2	3.9±0.6	9.0±0.6	20.3±5.4	9.1±3.7	1.3±2.0
NIDDM	51.8±26	69.7±6.4	78.6±7.1	1.6±0.9	3.5±0.9	3.5±0.9	23.4±16.2	19.1±15	8.0±10.2
NIDDM (Comp)	63.0±7	72.2±6.4	80.7±7.1	2.0±0.6	3.7±0.9	3.7±0.9	19.4±12.2	10.9±8.4	2.7±3.0

Lag Phase

LP was significantly lower in diabetics as compared to the controls with all the concentrations of collagen and archidonic acid used ($P<0.001$) table VI to IX).

Spontaneous Aggregation (SA)

SA was not observed in normal subjects. It was observed in 40 (40%) diabetics without complications and 47 (47%) patients with DR used ($P>0.001$).

DISCUSSION

Inspite of great advances in the treatment of DM, accelerated disease of the micro ciruclation accounts for the majority of blindness. Recent studies have improved our understanding of microvascular lesions[7,17].

Platelets have been implicated in the genesis of vascular lesions in diabetes mellitus[18]. Platelet hyper- aggregation have been reported in latent diabetes and in infants born to diabetic mothers[19,20]. This suggests that platelet aggregation is not the result but a cause of hyper aggregation. Stimulated platelets, release granular contents which also include platelet-derived growth factors (PDGF). These growth factors play an important role in the pathogenesis of proliferative retinopathy[10,11]. Our studies showed that it was enhanced in diabetic patients. The results of our study also revealed an increased intensity of aggregation and rate of aggregation. Our observations, however did not show

any significant difference between all the diabetic groups investigated. An increase in intensity and rate of aggregation was observed in other studies[17,21,22,23,24].

Another parameter of platelet hyperaggregation is spontaneous aggregation and reduced lag phase. In our studies we also observed spontaneous aggregation and reduced lag phase in all diabetic groups under observations, which correspond to other studies[18,22,23,24].

Since we did not find any difference between platelet aggregation patterns of diabetic patients with retinopathy and those without retinopathy, it may be suggested that platelet hyper aggregation observed in diabetic subjects are not the result of vasculopathy, but associated with diabetic state.

Hyperreactivity of the platelets results in the release of granular contents like PDGF which may be responsible for diabetic retinopathy. We conclude that platelet aggregation studies should routinely and periodically be performed in all diabetic patients. As platelet hyperaggregability plays a pivitol role in the development of diabetic retinopathy. The use of antiplatelet agent may help in preventing or delaying the onset of DR.

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