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COMPARISON OF PLATELET AGGREGATION RESPONSE IN TYPE 2 DIABETES AND NORMAL SUBJECTS

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ABSTRACT

Aims and objectives- To compare the platelet aggregation response in type 2 diabetes subjects and normal subjects. **Research methods**- 80 subjects with type 2 Diabetes Mellitus and 40 normal subjects were randomly selected. FPG, PPPG, HBA1c and platelet aggregation response were studied. **Results** - The mean age was 52.16±11.28 in the case group and 41.4±10.43 in the control group. There were 50 and 21 number of male subjects and 30 and 19 number of female subjects in the case and control group respectively. The mean fasting plasma glucose was 150.08±54.52 and 88.70±13.36 in the case and control group respectively. The mean post prandial plasma glucose was 233.98±82.99 and 129.95±15.27, mean HBA1c was 8.08±1.84 and 5.50±.42 in the case and control group respectively. The duration of diabetes ranged from newly detected diabetics to those with disease duration of more than 10 years. Platelet aggregation responses to ADP and epinephrine were measured and results were recorded as percentage for both groups. The difference between the case and control groups in the platelet aggregation responses induced was statistically insignificant. **Conclusions-** The platelet aggregation responses induced by ADP and epinephrine were similar in type 2 diabetes and normal subjects. So the routine use of anti platelet agents may not be necessary in all type 2 diabetes subjects for primary prevention of cardiovascular diseases. Anti platelet drugs can be used in secondary prevention and high risk patients. This is a small study done in a single centre. So a large multicentre study is necessary to prove this.

KEYWORDS: Anti platelet drugs can be used in secondary prevention and high risk patients.

INTRODUCTION

Platelets are small anucleate discoid cells that circulate in the bloodstream and participate in hemostasis. Their main function is to plug holes in blood vessel walls. Platelets do this by undergoing a change in shape, adhering to subendothelial surfaces, secreting the contents of intracellular organelles, and aggregating to form a thrombus in response to stimuli generated in endothelia of damaged blood vessels. These proaggregatory stimuli include thrombin, collagen, and epinephrine (which are exogenous to the platelet) and agents such as ADP, which is secreted from platelet storage granules, and thromboxane A_2(TxA_2), which is synthesized by the platelets during activation.[1] The pathogenesis of atherosclerosis in diabetes has several potential contributors, which include increased intravascular thrombin generation and reduced fibrinolytic potential.[2] Fibrinogen levels may also be elevated in diabetes.[3] which would contribute to fibrin clot formation and platelet aggregation. Fibrinolytic activity has been reported to be low in type 2 diabetes.[4] This is thought to be due to high levels of PAI-1, which inhibit the formation of fibrinolytic plasmin from plasminogen.

Endothelial injury or plaque rupture with platelet adhesion and aggregation at the site of injury may be the critical event in producing morbidity and mortality from atherogenesis because most coronary events occur with less than one-third narrowing of the vessel lumen.[5] Platelets may therefore assume an important role in the signal event in atherosclerosis in diabetes. This thesis is substantiated by the results of studies in which antiplatelet drugs such as aspirin and dipyridamole protected against stroke and myocardial infarction in both diabetic and nondiabetic individuals and also protected against diabetic retinopathy.[6] Evidences of abnormal platelet functions in diabetes mellitus have been shown as altered platelet functions.[7,8] increased aggregation of platelets that leads to acceleration of atherogenesis.[7] abnormal platelet activation suggested

to cause micro or macro angiopathies.[9,10] and platelet hyperactivities.[11,12] The aim of our study is to compare the platelet aggregation in type 2 diabetes subjects and normal controls.

METHODS

The study was performed at Karnataka institute of endocrinology and research over a period of 6 months. Informed consent was obtained from all the participants.

80 type 2 diabetes subjects and 40 healthy individuals were included in the study. Cases and controls did not have any other systemic disease. They were not on any drugs that interfere with platelet aggregation during the period of the study.

Fasting venous blood samples of all the participants were taken in vaccuated tubes. Plasma glucose was estimated by GOD POD method and HBA1c by HPLC method. Platelet aggregation responses to ADP and epinephrine were estimated by using platelet aggregometer.

Statistical Methods: Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. The following assumptions on data is made, Assumptions: 1.Dependent variables should be normally distributed, 2.Samples drawn from the population should be random, Cases of the samples should be independent.

Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more groups of patients, Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups. Pearson correlation is performed to find the correlation between variables.

Classification of Correlation Co-efficient (r)

Up to 0.1	Trivial Correlation
0.1-0.3	Small Correlation
0.3-0.5	Moderate Correlation
0.5-0.7	Large Correlation
0.7-0.9	V.Large Correlation
0.9- 1.0	Nearly Perfect correlation
1	Perfect correlation

Significant figures
+ Suggestive significance (P value: 0.05<P<0.10)
* Moderately significant (P value: 0.01<P ≤ 0.05)
** Strongly significant (P value: P≤0.01)

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 ,Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc**.**

RESULTS

The mean age was 52.16±11.28 in the case group and 41.4±10.43 in the control group. The sex distribution was as follows: males 50 and 21, females 30 and 19, in the case and control group respectively. The mean fasting plasma glucose 150.08±54.52 and 88.70±13.36 in the case and control group respectively. The mean post prandial plasma glucose was 233.98±82.99 and 129.95±15.27, mean HBA1c was 8.08±1.84 and 5.50±.42 in the case and control group respectively. The duration of diabetes ranged from newly detected diabetics to those with disease duration of more than 10 years.

Platelet aggregation responses to ADP and epinephrine were measured and results were recorded as percentage for both groups. The difference between the case and control groups in the platelet aggregation responses induced was statistically insignificant. Platelet aggregation responses were correlated with age, duration of diabetes and HBA1c and the differences were insignificant.

Study design: A comparative two group observational clinical study

Table 1: Age distribution of patients studied.

Age in years	Diabetics		Controls	
	No	%	No	%
<30	4	5.0	1	2.5
31-40	7	8.8	23	57.5
41-50	23	28.8	8	20.0
51-60	25	31.3	6	15.0
61-70	20	25.0	1	2.5
71-80	1	1.3	1	2.5
Total	80	100.0	40	100.0
Mean ± SD	52.16±11.28		41.40±10.43	

Mean age of diabetics are significantly more with P<0.001**

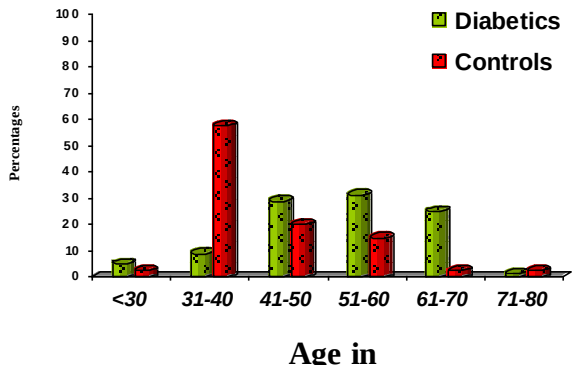

Table 1: Shows the age distribution of subjects studied ranging from 30 to 75 years. Maximum number of subjects are in the age group of 41 to 70 years.

Table 2: Gender distribution of patients studied.

Gender	Diabetics		Controls	
	No	%	No	%
Male	50	62.5	21	52.5
Female	30	37.5	19	47.5
Total	80	100.0	40	100.0

Samples are gender matched with P=0.293

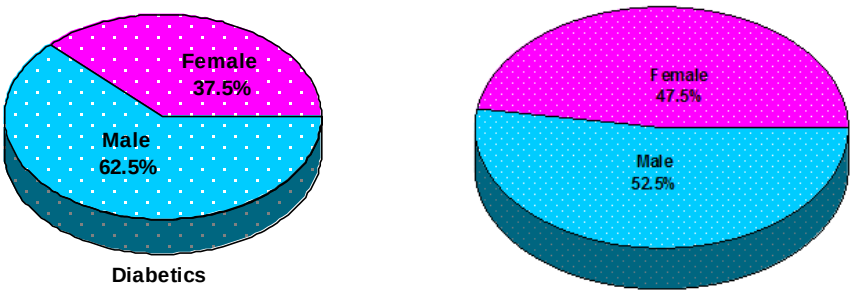

Table 3: Duration of diabetics in years.

Duration of diabetics	No. of patients	%
<1	15	18.8
1-2	12	15.0
2-5	14	17.5
5-10	23	28.8
10-20	16	20.0
Total	80	100.0

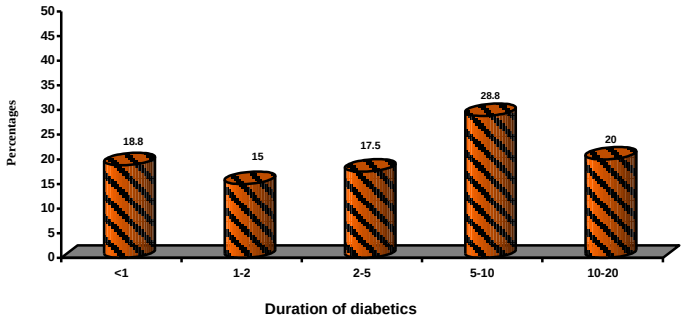

Table 3 shows that duration of diabetes range from new to >10 years. Maximum number of subjects are from 5 to 10 years.

Table 4: Comparison of plasma glucose parameters.

Glucose parameters	Diabetics (n=80)		Controls(n=40)		P value
	No	%	No	%	
FBS					
• <100	14	17.5	36	90.0	<0.001**
• 100-126	17	21.3	3	7.5	
• >126	49	61.3	1	2.5	
PPBS					
• <140	9	11.3	37	92.5	<0.001**
• 140-200	25	31.3	2	5.0	
• >200	46	57.5	1	2.5	
HbA1c					
• <6	5	6.3	37	92.5	<0.001**

• 6-12	72	90.0	3	7.5	
• >12	3	3.8	0	0.0	

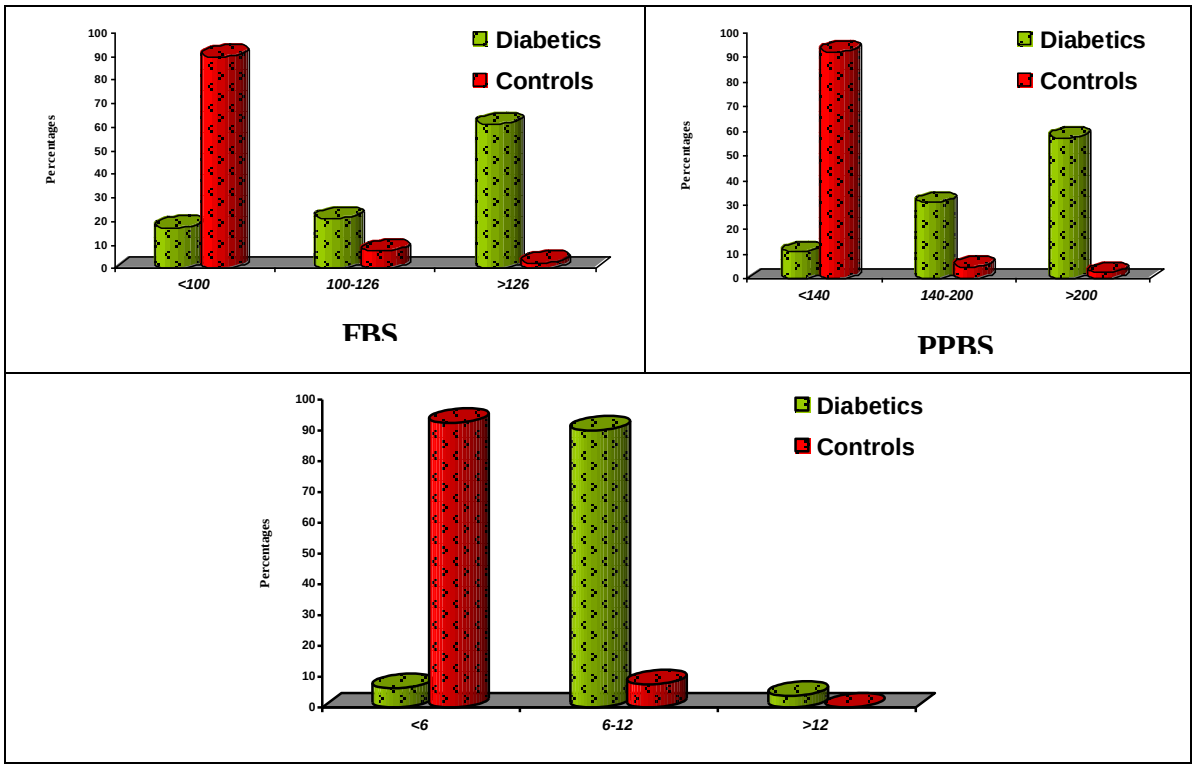

Table 5: Comparison of plasma glucose parameters in two groups studied

	Diabetics	Controls	P value
FBS	150.08±54.52	88.70±13.36	<0.001**
PPBS	233.98±82.99	129.95±15.27	<0.001**
HbA1c	8.08±1.84	5.50±0.42	<0.001**

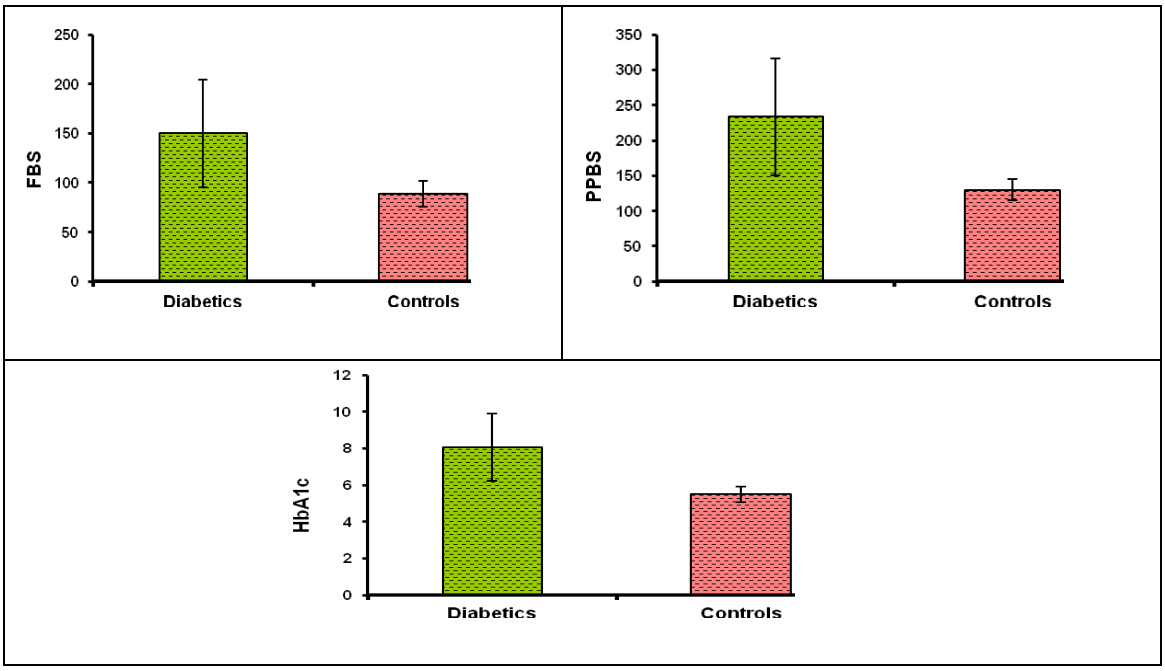

Table 6: Platelet aggregation response to ADP levels in two groups studied.

PLATELET AGG RESPONSE TO ADP	Diabetics		Controls	
	No	%	No	%
31-50	3	3.8	1	2.5
51-70	10	12.5	5	12.5
71-90	47	58.8	29	72.5
91-110	16	20.0	5	12.5
110-130	3	3.8	0	0.0
>130	1	1.3	0	0.0
Total	80	100.0	40	100.0
Mean ± SD	83.17±14.98		80.13±12.67	

Levels are statistically insignificant between two groups with P=0.273

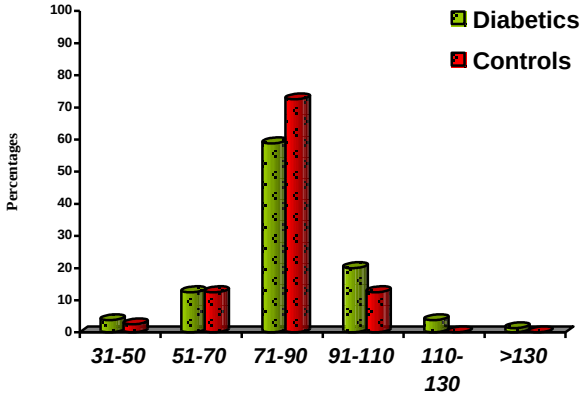

Table 7: Platelet aggregation response to EPINEPHRINE in two groups studied.

EPINEPH	Diabetics		Controls	
	No	%	No	%
<=10	5	6.3	4	10.0
11-30	10	12.5	3	7.5
31-50	7	8.8	2	5.0
51-70	6	7.5	3	7.5
71-90	39	48.8	24	60.0
>90	13	16.3	4	10.0
Total	80	100.0	40	100.0
Mean ± SD	67.27±28.91		68.60±29.47	

Levels are statistically insignificant between two groups with P=0.815

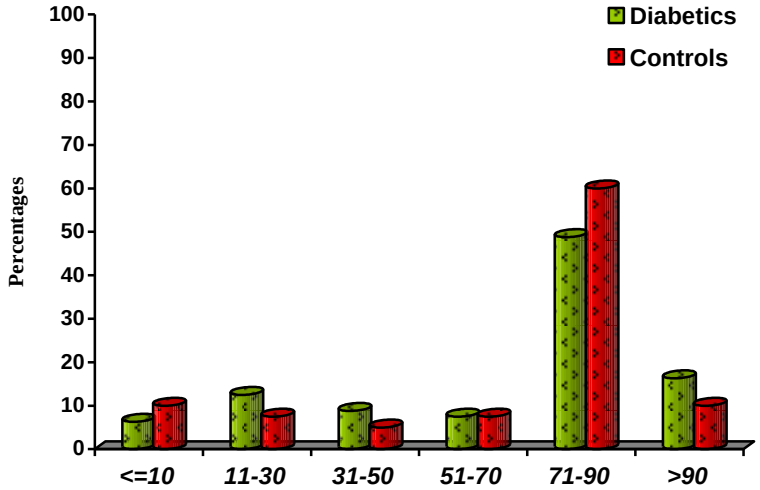

Table 8: Pearson correlation of study variables with age.

Pearson correlation	Group I		Group II	
	r value	P value	r value	P value
Age in years vs FBS	-0.186	0.098+	0.301	0.059+
Age in years vs PPBS	-0.170	0.132	0.114	0.483
Age in years vs HbA1c	-0.106	0.348	0.193	0.232
Age in years vs ADP	-0.068	0.550	-0.262	0.102
Age in years vs EPINEPH	0.081	0.473	-0.427	0.006**

Table 9: Comparison of study variables with age in years in cases.

	Age in years				P value
	≤40 years	41-50 years	51-60 years	>60 years	
FBS (mg/dl)	171.27±57.66	146.7±62.2	145.16±50.76	148.52±49.38	0.588
PPBS(mg/dl)	267.64±90.73	224.57±88.76	232.56±87.63	228.33±66.69	0.538
HbA1c	8.89±2.61	7.47±1.68	8.26±1.78	8.11±1.5	0.181
ADP	89.98±10.65	78.51±15.31	86.12±14.14	81.19±16.34	0.118
EPINEPH	72.92±27.46	60.93±31.1	67.17±28.26	71.37±28.65	0.592

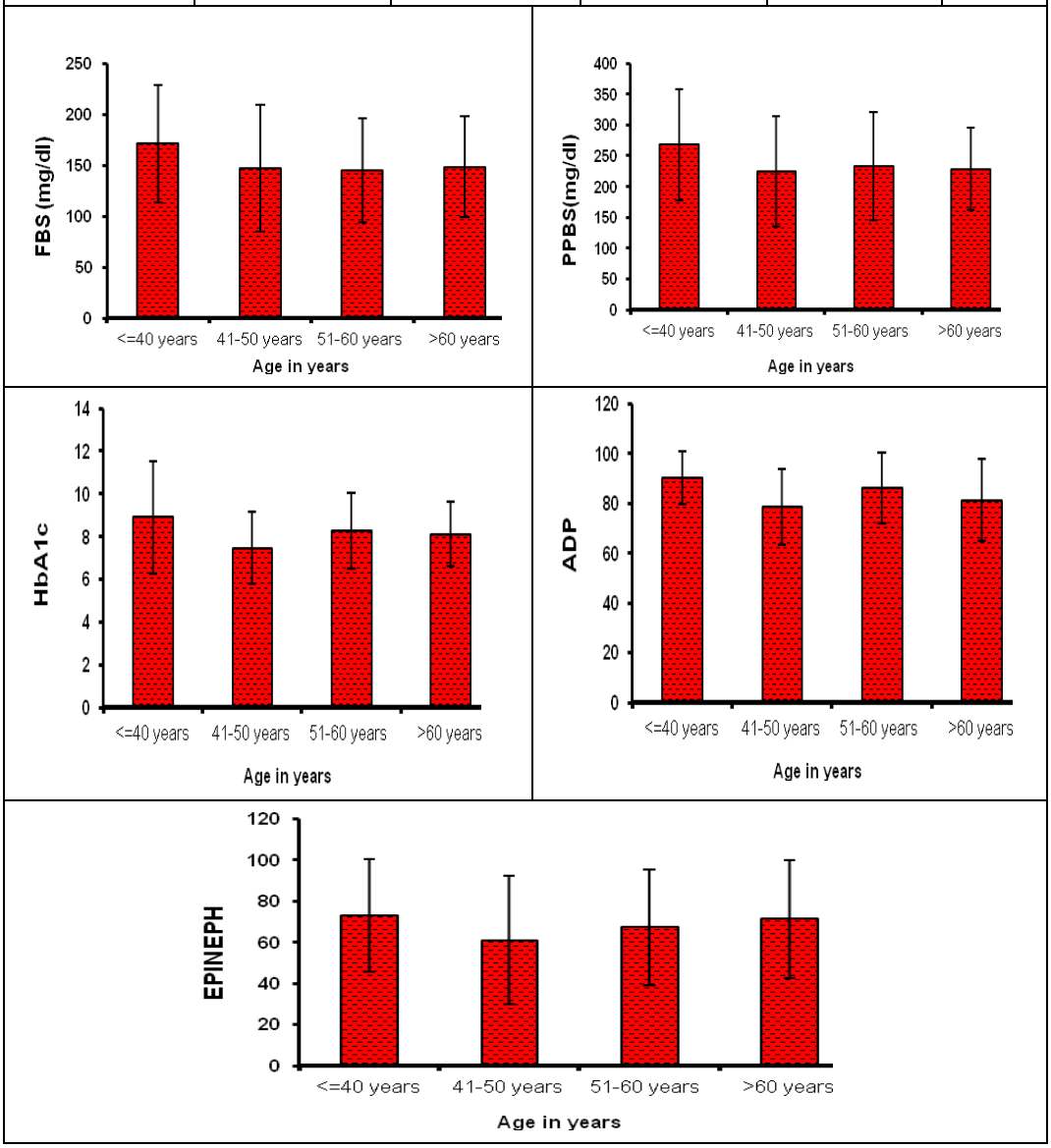

Table 10: Comparison of study variables with duration of diabetics.

	Duration of Diabetics (yrs)					P value
	≤1 years	1-2 years	2-5 years	5-10 years	>10 years	
FBS (mg/dl)	150.13±54.53	127±19.08	135.64±50.97	156.61±48.9	157.56±69.73	0.718
PPBS(mg/dl)	240.08±89	191±15.1	199.21±78.09	253.04±85.65	235.88±77.05	0.334
HbA1c	8.14±2.28	6.77±0.49	7.34±1.49	8.26±1.65	8.64±1.62	0.237
ADP	83.32±12.66	72.53±13.68	82.88±14.33	85.06±14.82	82.48±19.57	0.760
EPINEPH	69.37±29.1	51.03±41.03	54.39±29.24	71.74±27.29	72.01±27.89	0.309

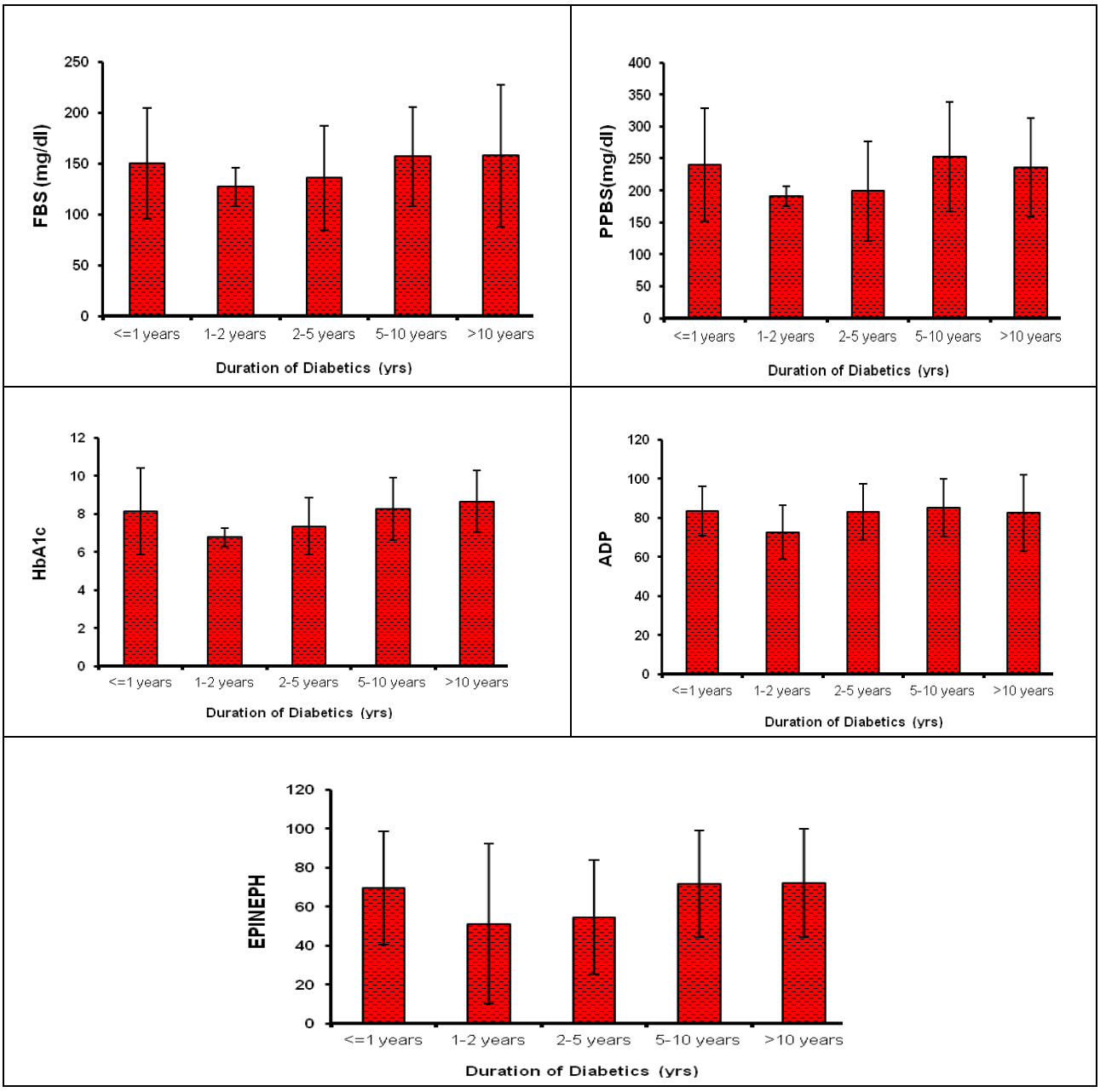

Table 11: Pearson correlation in of EPINEPH with glucose variables.

Pearson correlation	Group I		Group II	
	r value	P value	r value	P value
EPINEPH vs FBS	-0.140	0.214	-0.051	0.758
EPINEPH vs PPBS	-0.020	0.861	-0.068	0.672
EPINEPH vs HbA1c	-0.090	0.429	0.113	0.488

Table 12: Pearson correlation in of ADP with glucose variables.

Pearson correlation	Group I		Group II	
	r value	P value	r value	P value
ADP vs FBS	-0.152	0.179	-0.299	0.061+
ADP vs PPBS	-0.139	0.218	-0.071	0.663
ADP vs HbA1c	-0.050	0.660	0.276	0.085+

DISCUSSION

Platelet functions are significant to understand the pathophysiology of vascular disease in diabetes. The role of hyperglycemia is not clear in platelet hyperactivity in diabetic patients.[13] Platelet dysfunction may develop before vessel wall damage in diabetes.[14,15] Platelet dysfunction in diabetes including altered adhesion and aggregation is hypersensitivity to agonists.[16] Patients with type 2 diabetes had altered platelet functions and increased platelet aggregation responses with agonists.[17,18]

In this study ADP and epinephrine were used as activators and the platelet aggregation responses induced by both the activators were statistically similar.

Mean platelet volume is a marker of platelet function and activation. Larger platelets are more reactive and aggregable. Therefore it can be said that there is a relationship between platelet function and diabetic complications.[19-22]

Increase in HBA1c concentration indicative of worsening glycemic control accompanied by increase platelet volume and reflects deterioration of platelet function.[23]

In this study we have not found increase in platelet aggregability with increase in HBA1c. This is in agreement of the findings of F.M.K.Goven et.al,[24] Mandal et al,[25] and Hughes et al.[26]

In this study we did not find any relation between age and duration of diabetes with platelet aggregabality in type 2 diabetes subjects.

CONCLUSION

The platelet aggregation responses induced by ADP and epinephrine were similar in type 2 diabetes and normal subjects. So the routine use of antiplatelet agents may not be necessary in all type 2 diabetes subjects for primary prevention of cardiovascular diseases. Anti platelet drugs can be used in secondary prevention and high risk patients. This is a small study done in a single centre. So a large multicentre study is necessary.

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