Effect of Glycaemic control with Insulin Therapy on Platelet Aggregation in Type-2 diabetes Mellitus (NIDDM)

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SUMMARY

Coagulation abnormalities occur in the course of diabetes mellitus resulting in a state of thrombophila. Alterations in platelet function plays an important role in the genesis of cardiovascular and cerebrovascular disease. These alterations caused by hyperglycaemia are due to non-enzymatic glycation, the development of increased oxidative stress and decrease in levels of heparan sulphate and increased levels of fibrinogen. Besides antiplatelet drugs which have inherent antiplatelet activity, good metabolic control with hypoglycaemic agents has been shown to effect platelet activity. In this study, we have tried to demonstrate antiplatelet effect achieved by correcting hyperglycaemia with insulin in type-2 (NIDDM) patients.

INTRODUCTION

Patients suffering from diabetes mellitus have a high probability of developing myocardial infarction and cerebrovascular stroke accounting for most deaths in patients of Type-2 (NIDDM) diabetes mellitus [1]. The development of cardiovascular disease is clearly linked with the onset of a thrombotic event, as a result of marked thrombophilia [2, 3].

Alterations in glucose metabolism seem to be important cardivascular risk factors[4]. Non-diabetic women with an early cardiovascular event have been shown to have significantly elevated glycated haemoglobin levels [5]. Oxidative stress and increase in been considered significant cardiovascular risk factors [6, 7]. Thrombaphilic alterations have a pathogenetic value for cardiovascular events, oxidative stress being the common determining factor. Treatment with sulphonyureas like tolbutamide has been shown to be associated with a two fold increase in cardiovascular mortality as compared to insulin-treated patients as shown by the University Group Diabetes Programme (UGDP) [8]. This study is an endeavour to demonstrate effect of good metabolic control in type 2 (NIDDM) freshly detected diabetics on platelet aggregation with insulin therapy.

MATERIAL AND METHODS

Patient population

Ten freshly detected Type-2 (NIDDM) diabetic patients were randomly selected for this study which included 8 males and 2 females. Age of patients (mean SD) was 54.50 8.95 years, range (45-70 years).

Criteria for inclusion in the study were:

a) Stable freshly detected Type-2 diabetes mellitus diagnosed according to WHO criteria [9].
b) No history of drug intake particularly acetylsalicyclic acid, dipyridamole or alcohol in last four weeks.
c) No evidence of diabetic complications, coronary artery disease, major or minor vascular disease or infection.

All the patients underwent detailed history and thorough clinical examination besides investigations which included haemogram, blood urea, serum creatinine, calcium, potassium, blood urea, serum creatinine, calcium, potassium, sodium, phosphorus, alkaline phosphatase, proteins (Albumin/globulin), 24 hour urinary proteins, creatinine clearance, 12 lead basal electrocardiogram and blood glucose which was estimated by glucose oxidase method.

Design of the study

Fasting venous glucose and platelet aggregation was done in all the patients at the time of inclusion in the study, which comprised of the pre-treatment group or Group-1. All the patients diet was adjusted according to height, ideal body weight, daily habits and life style. Patients were asked to follow the adjusted diet strictly for 3 weeks. At the end of 3 weeks subjects were examined again, the fasting venous glucose and platelet aggregation studies were now started on intermediate acting insulin depending on their blood glucose levels to attain acceptable venous glucose. The insulin dose required ranged from (30-401U/day). Patients were

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now asked to continue the insulin regimens as advised continuously for 3 weeks. After 3 weeks venous blood glucose estimation was checked again to demonstrate acceptable control besides performing platelet aggregation studies, Group-III.

All the patients included in the study volunteered after proper consent and reported for follow-up at right time. Patients received insulin supply free of cost from Institute of Medical Sciences, Srinagar, where the study was performed after proper approval from ethical Committee.

Platelet aggregation

Platelet aggregation was determined by the standard method of Born [10], actual procedure being performed as employed by Sagel et al using ADP as aggregating agent in concentration of 1uM[11]. Any change in absorbency was equivalent to the percent of aggregation assuming that platelet poor plasma represented zero percent aggregation. The extent of aggregation at three minutes using ADP in concentration of 1uM, was recorded on the aggregometer.

RESULTS

This study on 10 freshly detected Type-2 diabetics (NIDDM) was conducted to determine effect of glycaemic control with insulin on platelet aggregation. Table-1 gives the details of weight, blood glucose and platelet aggregation before treatment , after dietary restriction and insulin therapy.

Table 1

Details of weight, venous blood glucose and platelet aggregation at different stages of study

		Venous blood	Platelet
Group	Weight (Kg)	Glucose mg/dl	Aggregation
	Mean ± SD	Mean ± SD	Percent (%)
Group-I*	63.05 ± 11.56	311.8 ± 74.84	66.47 ± 4.52
Group-II**	62.60 ± 12.03	276.60 ± 73.87	66.30 ± 3.20
Group-III***	58.60 ± 9.70	138.20 ± 20.44	59.47 ± 2.17

Group-I* = Pre-treatment group
Group-II** = Diet alone
Group-III* = Diet + Insulin

Table-2 gives detaliled statistical analysis of Table-1.

Table 2

Statisfical analysis of Table-1

Comparative	Weight	Venous	Platelet
		Blood glucose	Aggregation
Group-I VS. Group-II	P>0.5, NS	P<0.02, S	P>0.5,NS
Group-I Vs. Group-III	P>0.1,NS	P<0.01,HS	P<0.01, HS
Group-II Vs. Group-III	P>01., NS	P<0.01,HS	P<0.01,HS

NS = Not significant
S = Significant
HS = Highly significant

When Group-1 parameters were compared with Group-11 parameters no significant change was observed in weight and platelet aggregation of patients, however, significant change in blood glucose levels were noted. However, these blood sugars were no where near the acceptable values. Comparing Group-1 vs. Group-III and Group-II vs. Group-III, no significant change in weight had been observed but there had been a highly significant decrease in blood glucose and platelet aggregation.

DISCUSSION

A well established correlation exists between development of macrovasclar and microvascular disease in diabetes mellitus [1]. Alteration in glucose metabolism now seems to be an important cardiovascular risk factor as shown by the Framingham study. This study showed that non-diabetic women with an early cardiovascular event had significantly elevated glycated haemoglobin levels [5]. The development of cardiovascular disease in diabetics is clearly linked with onset of marked thromboxane A-2 has been seen in Type-2 diabetics which reversed with better metabolic control [12]. Increased oxidative stress with resultant increased production of free radicals is linked to process of glucose auto-oxidation and to the possibility that glycated proteins themselves release free radicals. Production of these free radicals is directly correlated to metabolic control and more directly to hyperglycaemia [13, 14]. Reduced synthesis of heparan sulphate, which regulates pericellular thrombotic phenomenon at the level of endothelial membrane, is linked with increased platelet aggregability (thrombophilia) in diabetes mellitus [15, 16].

Several studies have demonstrated enhanced plafelet aggregation in platelet rich plasma of some patients with diabetes mellitus [17 – 19]. Platelets from patients with diabetes mellitus have been shown to have increased in-vitro aggregability to a variety of agents[20]. Sagel et al demonstrated platelet aggregation in platelet rich plasma of four Type-2 diabetes mellitus patients to ADP (1uM) as 64.0% 6.0% [11], while in our study basal platelet aggregation to same concentration of ADP has been 66.47% 4.52%. Thrombophilic alterations have strictly been correlated with the poor regulation of glucose metabolism, these alterations have pathogenetic value for cardiovascular events[4]. Oral hypolycaemic agents especially tolbutamide have been shown to have decreased mean percentage platelet aggregation in laboratory conditions from 64.0% 6.0% to 21.0% 3.0% independent of its hypoglycaemic activity[11].

Glicalazide, a second generation sulphonylurea has been shown to have an anti-aggregation property by causing release of plasminogen activator from the vessel wall and inhibiting activity at different stages of thrombus formation[21].

In our study, when Group-1 parameters were compared to Group-11, no significant difference in weight as well as platelet aggregation was observed, however, a significant change was observed in blood glucose levels, Blood glucose in Group-11 was nowhere near the acceptable range, even though they had significantly dropped from Group-1. When Group-1 and Group-11 parameters were compared to Group-III, we found that there was a

Highly significant decline in blood glucose levels which was simultaneously associated with a highly significant decline in platelet aggregation as well ($P < 0.01$). Changes in platelet function during intensive insulin treatment have been shown to be as a direct effect of changes in plasma glucose, insulin or due to secondary changes in different metabolites or hormones[22]. A physiological increase in the plasma insulin levels has been reported to directly inhibit ADP induced aggregation[23].

This study suggests that besides correcting hyperglycaemia, which has direct effect on platelet aggregation by decreasing oxidative stress and fibrinogen levels, insulin directly effects platelet aggregability.

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