Research Article

Evaluation of plasma fibrinogen, D-dimer and C-reactive protein as predictive parameters for coronary heart diseases in type 2 diabetic patients

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Abstract

Background: Disordered hemostatic mechanisms can play a role in the pathogenesis of macrovascular complications in type 2 diabetes either by leading to hypercoagulable state, or as circulating markers for arteriosclerosis.

Aim: To evaluate the plasma levels of fibrinogen, D-dimer and C-reactive protein in patients with type 2 Diabetes mellitus (DM) and find a relation with disease duration, diabetic control and ischaemic heart disease (IHD).

Patients and methods: Sixty adults attending the National Diabetes Center at Al-Mustansiriya University were randomly selected and divided into 3 groups, 20 patients each. Group 1 and 2 consisted of type 2 diabetic patients without and with history of ischemic heart disease, respectively. Group 3 included 20 healthy controls. Blood was collected for measurement of fibrinogen, D-dimer, C-reactive protein and HbA_1C.

Results: Mean plasma D-dimer and CRP but not fibrinogen were significantly higher in group 1 and 2 compared to the control group, in which they were significantly higher in group 2 than group 1.

CRP and fibrinogen but not D-dimer levels were positively correlated with HbA_1C levels in the diabetic groups. The levels of plasma fibrinogen, D-dimer and CRP were significantly increased in diabetic patients with increase in the disease duration. Only levels of CRP had a significant relation with the history and type of ischemic heart disease.

Conclusions: Hyperglycemia, insulin resistance and chronic inflammatory stress may be the causative factors for abnormal levels of hematological markers in type 2 DM. Their effect is augmented by the duration of DM and the association with IHD.

Keywords: Male infertility, Azoospermia, Testicular biopsy, Histopathology

INTRODUCTION

Patients with diabetes mellitus have an increased risk of cardiovascular diseases, especially myocardial infarction, cerebrovascular and peripheral vascular diseases [(1)]. Coronary artery diseases (CAD) are the most frequent cause of mortality in diabetic patients; especially type 2 diabetes; men with diabetes have 2-4 folds more CAD mortality risk than non-diabetic men at any level of serum cholesterol [(2)]. Much attention has been devoted to factors of altered haemostatic balance like abnormalities in platelet function, increased blood coagulability and altered fibrinolytic system in the pathogenesis of cardiovascular morbidity and mortality in these patients [(1)]. In diabetic patients, the disturbances in hemostasis leading to accelerated fibrin formation (hypercoagubility) and

delayed fibrin removal may contribute to the development of atherosclerosis [3].

The aim of the study is to evaluate the plasma levels of fibrinogen, D-dimer and C-reactive protein in patients with type 2 Diabetes mellitus (DM) and find a relation with disease duration, diabetic control and ischaemic heart disease (IHD).

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PATIENTS AND METHODS

A case-control study was conducted on forty patients with type 2 diabetes who had attended the National Diabetes Centre, in Al-Mustansirya University between April and June 2012, along with twenty healthy age matched control subjects (group 3). The diabetic patients were presented at different durations of illness. The patients were arranged in two groups; group 1: comprised of twenty diabetic patients without history of ischemic heart disease (11 male, 9 female), and group 2: comprised of twenty diabetic patients with history &/or ECG changes of ischemic heart disease (12male, 8 female). Table 1 summarizes the age, sex and disease duration distribution among the three groups.

5 ml of blood was obtained from patients and control subjects, from it 2 ml of blood was added to Ethylenediaminetetra-acetic acid (EDTA) tube for HbA1c estimation, 1.8 ml was added to 0.2 ml trisodiumcitrate tube for fibrinogen and D-dimer assay, and finally the serum obtained from the remaining 1 ml of blood was used for CRP assay.

Fibrinogen assay was performed using Clauss technique [4] with a commercially available kit from Croma test©, Linear chemicals, Spain. CRP test was performed using a commercially available kit from Croma test©, Linear chemicals, Spain; by testing a suspension of latex particles coated with anti-human CRP antibodies against unknown serum [5]. Plasma D-dimer was assessed using a commercially available kit (D-Di test, Stago, France).The latex particles provided in the D-dimer test were coated with mouse anti-human D-dimer monoclonal antibodies[6]. HbA_1C was measured using a commercially available kit (Stanbio glycohemoglobin pre-fil, Stanbio, USA) [7].

Computerized statistical analysis were performed using SPSS 2007.The numeric data expressed as mean±SD and nominal data expressed as number and percent. The numeric data was analyzed using student t-test (P-value < 0.05 is considered statistically significant difference), correlation coefficient was used to study the strength of association between two variables.

Table 1. Age, sex and disease duration distribution among the three groups included in the study.

Age	DM without IHD (group 1)	DM with IHD (group 2)	Control group (group 3)
40-49 yeas	15%	15%	45%
50-59 yeas	65%	55%	40%
60-69 yeas	20%	30%	15%
Mean age (years) ± SD	54.06±5.3	56.28±5.3	51.72±5.8
Males	55%	60%	60%
Females	45%	40%	40%
Duration of DM ± SD	6.8±3.2	6.6±2.8	-
Duration of IHD ± SD	-	4.1±2.5	-

RESULTS

As shown in figure 1, there was a statistically significant increase in the levels of plasma D-dimer and highly significant increase in CRP levels in both diabetic groups when compared to the controls. The levels of fibrinogen were slightly higher in the diabetic patients but not to a statistical significance as compared to the control group. HbA_1C was highest in patients of group 1 but not to a statistical significance as compared to group 2.

The results showed there is no significant effect of age on fibrinogen levels in any of the three groups, as shown in table (2).Also there was no significant effect of sex on hematological parameters in any of the three groups, as shown in table (3).

There was a weak positive correlation between plasma levels of fibrinogen and CRP (but not D-dimer) on one hand and the level of HbA_1C in diabetic patients regardless of association with IHD, as shown in table (4). However, the level of plasma fibrinogen, D-dimer and CRP were significantly higher in diabetic patients (regardless of the history of IHD) with history of DM for ≥ 5 years than those

having DM for < 5 years (Table 5). Regarding IHD, This study showed that only CRP was significantly high in patients with MI compared to patients with angina, while there were no significant differences in fibrinogen and D-dimer levels among those patients (Table 6).

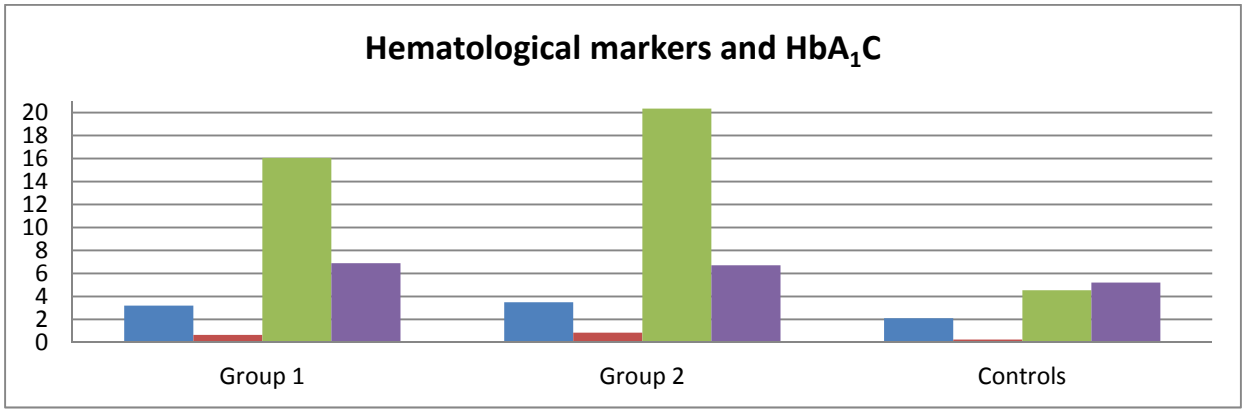

Figure 1: Hematological markers and HbA_1C in diabetic groups compared to the controls (*: statistical significance, **: high statistical significance).

Table 2: effect of age distribution on fibrinogen levels.

	Age < 60 (n= 30)	Age ≥60 (n=10)	p-value
Fibrinogen level in diabetics without IHD	2.90±0.6	3.15±0.4	0.602
Fibrinogen level in diabetics with IHD	3.16±0.52	3.54±0.03	0.271
Fibrinogen level in control group	2.10±0.31	2.53±0.52	0.938

Table 3: effect of sex difference on hematological & biochemical parameters.

	Parameter	**Sex**		**P-value (t-test)**
		Male	**Female**	
Group 1 DM without IHD	D-dimer (µg/ml)	0.61±0.3	0.68±0.5	0.447
	CRP (mg/l)	12.4±1.0	20.3±1.1	0.368
	Fibrinogen (g/l)	2.77±0.48	3.48±0.81	0.216
	HbA1c	6.9±0.6	6.9±0.7	0.746
Group 2 DM+IHD	D-dimer (µg/ml)	0.81±0.05	0.88±0.03	0.869
	CRP (mg/l)	17.02±1.2	22.4±0.8	0.395
	Fibrinogen (g/l)	3.29±0.63	3.75±1.01	0.104
	HbA1c	6.4±0.7	6.9±0.8	0.809
Control	D-dimer (µg/ml)	0.23±0.06	0.24±0.2	0.678
	CRP (mg/l)	4.0±0.50	4.89±0.6	0.410
	Fibrinogen (g/l)	2.0±0.48	2.05±0.46	0.774
	HbA1c	5.2±0.5	5.4±0.5	0.764

Table 4: Relation between HbA1c and (fibrinogen, D-dimer & CRP) in each group.

	HbA1c	Fibrinogen	R value of fibrinogen	D-dimer	P-value of D-dimer	CRP	P-value of CRP (t-test)

Group 1 (DM without IHD)	6.9±0.7	3.2±0.84	0.385*	0.65±0.05	0.326	16.05±0.5	0.035
Group 2 (DM with IHD)	6.7±0.8	3.5±0.21	0.485*	0.84±0.11	0.206	20.35±0.65	0.044
Group 3 (control)	5.2±0.5	2.1±0.48	0.129**	0.24±0.07	0.831	4.54±0.2	0.736

* Weak +ve correlation. ** No correlation.

Table 5: The effect of diabetes duration on the hematological markers expressed as mean±SD.

Duration of Disease	Parameter	Duration <5 years	Duration ≥5 years	p-value (t-test)
DM without IHD	D-dimer (µg/ml)	0.47±0.4	0.76±0.6	0.031
	CRP (mg/l)	10.69±1.2	20.50±2.1	0.016
	Fibrinogen (g/l)	2.1±0.17	3.44±0.56	0.046
DM with IHD	D-dimer (µg/ml)	0.53±0.05	1.03±0.02	0.040
	CRP (mg/l)	12±0.6	24.3±1.0	0.001
	Fibrinogen (g/l)	3.13±1.04	3.94±0.82	0.038

Table 6: The effect of IHD type on the hematological markers expressed as mean±SD.

Parameter	Type of IHD		p-value (t-test)
	Angina (stable &unstable)	MI	
Fibrinogen	3.04±0.81	3.9±1	0.104
D-dimer	0.81±0.2	0.87±0.1	0.608
CRP	17.43±0.4	26.6±0.6	0.027

DISCUSSION

In this study, the mean of fibrinogen level was higher in group 1 & 2 patients compared to control group but this difference did not reach the level of significance. However; the mean levels of plasma fibrinogen were nearly comparable between groups 1 & 2. This increase in fibrinogen in group 1 & 2 compared to control group was in agreement with many studies [8, 9, 10, 11, 12] which suggested that hyperglycaemia and/or insulin resistance will increase intracellular oxidative stress, leading to elevated fibrinogen level in type 2 DM. Moreover; this variation in plasma fibrinogen level in type 2 DM patients may be attributed to alteration in fibrinogen turnover which may had preceded clinical diabetic micro- or macro-vascular complications, thus this variation in fibrinogen level may potentially contribute to the onset of the vascular complications.

The significant elevation of D-Dimer in diabetic patients with or without IHD in comparison to the control group, suggests that, in diabetic patients, the increase in the coagulation pathway markers is more pronounced than the changes in the fibrinolysis pathway, and this result in increased level of D-dimer in diabetic patients [13, 14].

The significant elevation of CRP in diabetic patients with or without IHD in comparison to the control group and the significant elevation of CRP in type 2 diabetic patients with IHD compare to that of patients without IHD may be explained by the fact that inflammation may had play a role in the pathogenesis of type 2 diabetes, thus patients with high level CRP are more liable to develop type 2 DM and its complications [15, 16].

The weak positive correlation between fibrinogen and HbA1c indicates that a good glycaemic control can reduce the level of fibrinogen and reverse its abnormality, possibly by limiting its turnover [17].

The significant relationship between CRP and HbA1c in diabetic patients with IHD and without IHD may be attributed to hyperglycaemia which is usually associated with systemic inflammation that results in an increase in CRP level in diabetic patients [18].

Prolonged DM duration is associated with increased insulin resistance and in turn result in increase fibrinogen

turnover, which explains the association of diabetes duration with hematological markers variation in the current study and in other study [19].

The study also showed significant elevation of D-dimer in diabetic patients with increasing duration of the illness, which might be due to increasing state of hyperglycaemia and oxidative stress with prolonged duration of DM, and this result in wide-spread vascular complication of DM, as shown in other studies by Nwose *et.al.*[20] & Kafle *et. al.* [21].

The significant increase of CRP levels with duration of diabetic illness indicates that more inflammatory process is associated with longer duration of the disease with more vascular complication.

In this study, there was a significant elevation of CRP in patients with MI than those with angina pectoris and this might indicate the severity of the inflammatory process in these conditions as seen in other study done by Terrance *et al.* [22]

Conclusion

Hyperglycemia and/or insulin resistance in type 2 diabetes mellitus is associated with abnormal changes in the levels of hematological markers including fibrinogen, D-dimer and CRP. These changes are directly proportional to the duration of the diabetes, the severity of hyperglycemia as indicated by the levels of HbA1c and future risk of having vascular complications like coronary artery diseases.

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