Activity assay of plasma fibrinogen in patients with diabetes.

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Abstract:

Thrombosis secondary to atherosclerosis or hypercoagulability state in diabetes mellitus may be the cause of death in many patients. Patients with diabetes mellitus (n=28, 16 males and 12 females), together with 28 age and sex matched healthy individuals (as controls), were studied after their consent. For each individual (patients and controls), following medical history,
4 ml of venous blood sample was obtained and investigated for HbA1c level, fibrinogen level by doing Claus technique and blood sugar estimation. Hyperfibrinogenaemia in patients with diabetes mellitus (mean plasma fibrinogen 6.8±1.1) is statistically significant (P< 0.05) as compared to control (mean plasma fibrinogen 3.3±1.3). Plasma fibrinogen level is high in patients with diabetes mellitus, suggesting that plasma fibrinogen can contribute to vascular diseases in patients with diabetes mellitus like vascular thrombosis.

تقييم نشاط الفايبرينوجين في بلازما الدم لدى المرضى المصابين بداء السكري.

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خلاصة البحث :

خثرة الدم بسبب تصلب الشرايين او بسبب زيادة قابلية الجسم للتخثر الدموي يمكن ان يكون سببا للوفاة في المرضى المصابين بداء السكري. تمت دراسة 28 شخصا (16 من الذكور ؛ 12 من الاناث) مع دراسة 28 شخصا من نفس الجنس والعمر من الاصحاء للمقارنة بعد اخذ الموافقة منهم. تم سحب 4 مليليتر من الدم الوريدي من كل شخص وتم اجراء الفحوصات المختبرية الخاصة بنسبة الهيموغلوبين نوع أي وان سي الخاص بنسبة السكر التراكمي في الدم بالطريقة الكيميائية وقياس نشاط او فعالية الفايبرينوجين بطريقة التخثر باستخدام مادة الثرمبين لاجراء الاختبار بطريقة كلاوس العالمية و قياس نسبة السكر في الدم بالطريقة الكيميائية.تبين ان نشاط او فعالية الفايبرينوجين في الدم عالية بفرق احصائي مهم لدى المرضى المصابين بداء السكري(6,8±1,1) مقارنة بالاصحاء(3,3±1,3) لذا فان نشاط او فعالية الفايبرينوجين عالية لدى مرضى داء السكري ويمكن ان يسبب او يساهم مع بقية العوامل في تخثر الدم في الاوعية الدموية.

Introduction

Fibrinogen is a 340 KD (Killo-dalton) molecular weight plasma protein[1] that circulates at concentration of 2-4 g/L with a plasma half life of four days and is considered to be very important for both intrinsic and extrinsic coagulation pathways since the last step of cascade coagulation systems includes the conversion of fibrinogen to fibrin i.e clot formation which is stabilized by activated factor XIII[2]. It is the coagulation factor number one which is synthesized by the liver cells (hepatocytes) under the control of genes located on chromosome number four[3-5]. Biological activity of fibrinogen is determined by measuring thrombin time, a laboratory test which measures the time needed for the conversion of in vitro plasma fibrinogen to fibrin clot with subsequent matching of this time with plasma fibrinogen activity[6]. A study showed that thrombotic factors (smoking, low fruit and vegetables intake, high fibrinogen and high homocysteine) are important risk factors in the development of coronary heart diseases[7] which are the causes of death in about 50% of type 2 diabetes mellitus and about 25% of type 1[8]. Hyperfibrinogenaemia in patients with diabetes mellitus is an additional essential risk factor for the development myocardial infarction or stroke through inducing atherosclerosis of blood vessel walls or inducing thrombus formation because patients with diabetes, especially after the development of complications, are predisposed for both atherosclerosis and thrombosis with or without the presence of hyperfibrinogenaemia[9]. The exact mechanism of development of thrombosis in patients with diabetes mellitus who have hyperfibrinogenaemia is not completely understood; however, studies showed that high plasma fibrinogen plays important role in inducing thrombosis in two ways. The first way is by increasing blood viscosity i.e reducing blood flow and the second way is by increasing the number of fibrinogen receptors on platelet membrane leading to platelet aggregation and subsequent stimulation of coagulation cascade[10-12]. Another study showed that the thrombotic activity of fibrinogen is enhanced in the presence of high blood sugar in both type 1 and type 2 diabetes mellitus[13]. Plasma fibrinogen levels are also elevated following acute myocardial infarction[14] and stroke in the first 24 hours of the disease[15].

Patients and methods

From April 2012 to January 2013, 28 patients with diabetes mellitus (16 males and 12 females with age range of 33-62 years), together with 28 age and sex matched healthy individuals (as controls) who attended a private legal laboratory specialized for hematological investigations (Zaid Bin Ali medical laboratory/karbala city/Iraq) were studied after their consent.

 For each individual (patients and controls), following medical history, 4 ml of venous blood sample was obtained and investigated as follows:

Two ml of blood were transferred to EDTA tube for HbA1c assay using the commercially available kit (Glycohemoglobin-Human GMBH-65205 Wiesbaden-Germany) by doing the spectrophotometric evaluation, 0.9 ml of blood were transferred to 0.1 ml sodium citrate containing plastic tube for plasma fibrinogen assay (clotting assay) using the commercially available strong thrombin solution (BIO-FIBRI-BIOLABO U.S.A), by doing Claus technique[16] and the remaining 1.1 ml of blood was used for blood sugar estimation using the commercially available kit (Randox laboratories limited 55 Diamond road Crumlin, country Antrim U.K BT 29 4Qy). The normal range of plasma fibrinogen is 2-4 g/L, normal HbA1c level is 4.2%-6.2% . and normal random blood sugar is 80-180 mg/dl. Statistical analysis is done using the SPSS program (version 17). P value below 0.05 was considered to be statistically significant.

Results

Male to female ratio among diabetic patients was 1.3:1. Age range was 33-62 (mean 38.3). Table 1 shows the statistical significance of different mean values in both diabetic patients and the control group. Table 2. Shows the statistical significance of hyperfibrinogenaemia in both diabetic patients and the control group regarding age, sex, somking, random blood sugar and HbA1c. Hyperfibrinogenaemia in patients with diabetes mellitus (mean plasma fibrinogen 6.8±1.1) is statistically significant (P< 0.05) as compared to control (mean plasma fibrinogen 3.3±1.3) while no statistically significant difference was found between the mean plasma fibrinogen level in male diabetic patients (mean: 6.8±1.4) and female patients (mean: 6.5±1.5). Out of 28 (100%) cases of diabetes mellitus, 12 patients (42.86%) were smokers (smoking 20-30 cigars per day) while 16 diabetic patients (57.14%) were not smokers. The mean plasma fibrinogen level in diabetic patients who are smokers was (7.5±1.3) which is significantly higher than the mean of non smokers diabetic patients (4.9±1.2). Out of 28 control individuals, 10 (35.7%) were smokers and 18 (64.3%) were non smokers. The mean plasma fibrinogen level in smokers of control group was (6.1±1.2) which is significantly higher than the mean of non smokers in the control group (2.9±1.0).

The mean value of HbA1c of 28 (100%) diabetic patients was 7.7±1.2, range 5.8-10.0%. Pearson's coefficient of correlation (r) between peak levels of plasma fibrinogen and HbA1c in diabetic patients was 0.49 i.e the higher HbA1c, the higher plasma fibrinogen level. Similarly, levels of random blood sugar were positively correlated (r=0.49%) with levels of plasma fibrinogen in diabetic group with a mean random blood sugar of (231± 59) while no correlation was found in the control group regarding both HbA1c (mean 5.1 ±0.9) and random blood sugar (mean 116±12)

Discussion

The study showed that the mean plasma fibrinogen level in diabetic patients (6.8± 1.1) is significantly higher than that of control (3.3±1.3). Mittal S, Ashutosh Dwivedi RN, et al study showed similar correlation[17]. Another three studies showed that plasma fibrinogen is significantly higher in diabetic patients than controls regardless duration and type of disease[18&19] (i.e both type 1 and type 2 diabetes have similar rise in plasma fibrinogen levels). The exact causes of hyperfibrinogenaemia in patients with diabetes are unknown; however, studies showed that there is a rise in procoagulants in diabetic patients[8]. Procoagulants are proteins that induce serial activation of clotting factors starting from activation of factor number XII and ending in activation of clotting factor number one (fibrinogen)[2]. So that, it is expected to find an increase in plasma levels of other clotting factors in diabetic patients. A study showed that there is an increase in factor VII, VonWillebrand's factor and lipoprotein (a) in patients with diabetes. Lipoprotein (a) is has a major effects in diabetic patients by inhibiting plasminogen, decreasing fibrinolysis and relatively increasing fibrinogen activity[20]; however, studies showed that the increase in plasma fibrinogen levels rather than the other clotting factors is the major causative agent of vascular atherosclerosis[21] The mean plasma fibrinogen level in diabetic patients who are smokers was significantly higher (7.5± 1.3) than that of diabetic non smokers (4.9± 1.2). Similarly, in the control group, the mean plasma fibrinogen level in smokers (6.1± 1.2) was significantly higher than that of non smokers (2.9±1.0) which suggests that smoking is a major risk factor for thrombosis in both diabetic and non diabetic individuals directly or indirectly via causing hyperfibrinogenaemia. P Mistry et al study showed similar results[15]. Education of peoples about such effects of smoking is very important to prevent the development of hyperfibrinogenaemia in non diabetic individuals and prevent further rise of plasma fibrinogen in diabetic patients. The study showed that there is a positive correlation (r=0.49) between plasma fibrinogen levels on a hand and levels of HbA1c and random blood sugar

in diabetic patients on another hand i.e the higher the HbA1c level and random blood sugar, the higher the plasma fibrinogen level and suggests that the control of blood sugar in diabetic patients will be followed by a control in plasma fibrinogen levels. There is a study which showed similar positive correlation between plasma fibrinogen levels and glycemic control in diabetic patients[22].

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Table1. Mean values of HbA1c, randome blood sugar, plasma fibrinogen, and number of smokers in both diabetic patients and the control group.

Parameters	Diabetic patients	Control group	Statistical significance
mean HbA1c%	7.7±1.2	5.1±0.9	*P<0.05
mean random blood sugar in mg/dl	231±59	116±12	*P<0.05
mean plasma fibrinogen in g/L	6.8±1.1	3.3±1.3	*P<0.05
number of smokers	12/28(42.86%)	10/28 (35.7%)	**P>0.05

NB: *: P<0.05(significant), **: P>0.05 (not significant).

Table 2. Significance of hyperfibrinogenaemia in both diabetic patients and the control group regarding age, sex, somking, random blood sugar and HbA1c.

Parameters	Diabetic patients	Control group
Age in years	33-62**	33-62**
Sex	16 males& 12 females**	16 males& 12 females**
Smoking	12/28 (42.86%)*	10/28 (35.7%)*
mean random blood sugar in mg/dl	231±59***	116±12****
mean HbA1c%	7.7± 1.2***	5.1± 0.9****

NB: *: P<0.05(significant), **: P>0.05 (not significant)., ***: positive correlation(r=0.49), ****: no correlation.