Antiphospholipid Antibodies in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Patients with diabetes mellitus type 2 have a greater risk of accelerated atherosclerosis. Antiphospholipid antibodies(aPL)are associated with greater risk for thrombosis. To demonstrate the possible role of anticardiolipin(aCL) and anti-β2 glycoprotein(β2 GP1) antibodies in such patients, we investigate the presence of these antibodies in a group of type 2 diabetic patients.

Objectives: 1. To investigate the presence of anticardiolipin IgM and IgG antibodies and anti-β2 GP1 IgG antibodies in diabetic patients and compare them with a control group.

2. To analyze their potential implication in the occurrence of vasculopathy in such patients.

Patients and Methods: Fifty patients with type 2 diabetes mellitus and 33 healthy subjects were included in the study. Each blood sample was tested for IgM, IgG aCL antibodies and for anti-β2 GP1 IgG antibodies.

Results: Seven patients were positive for aCL IgM, 6 were positive for aCL IgG and 4 patients were positive for anti-β2 GP1 IgG antibodies. There was no differences in the means of IgM, IgG aCL and anti-β2 GP1 IgG antibodies titers in patients with complicated and uncomplicated diabetes mellitus.

Conclusion:

The aCL and β2 GP1 antibodies positive titer means among type2 diabetics were significantly higher than non-diabetic subjects.

Positive but low titers of aCL and β2 GPI antibodies could suggest that these autoantibodies may play a minor role in the pathogenesis of atherosclerosis.

Low titers of aCL and β2 GPI antibodies were seen in complicated and non-complicated diabetic populations that probably lessen the importance of these autoantibodies as effective contributors in the pathogenesis of diabetic vasculopathy.

Keyword: Diabetes, antiphospholipid antibodies, anticardiolipin antibodies, vasculopathy.

الاجسام المضادة للشحوم الفوسفاتية لدى مرضى السكري من النوع الثاني

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الخلاصة

الهدف من الدراسة: المرضى الذين يعانون من مرض السكري من النوع ٢ لديهم خطورة كبيرة للاصابة بتصلب الشرايين . يرتبط وجود أضداد الشحوم الفوسفاتية مع خطر حصول التخثر. ولإثبات تاثير وجود اضداد الكارديولبين واضداد β 2GP 1 لدى هؤلاء المرضى تم التحري عن وجود هذه الأضداد في مجموعة من مرضى السكري من النوع الثاني.

١. للتحري عن وجود اضداد الكارديولبين نوع وIgM IgG واضداد β 2GP 1 نوع IgG في مرضى السكري ومقارنتها بمجموعة الغير مصابين بالسكري.
٢. لتحليل التأثيرات المحتملة لوجود هذه الاضداد في حدوث الاعتلال الوعائي لدى هؤلاء المرضى.

المرضى وطرائق العمل: شملت الدراسة خمسين مريضاً مصابا بالنوع الثاني من داء السكري و ٣٣ شخصا سليما من المجموعة الضابطة. تم جمع عينات دم من المجاميع المذكورة من اجل الكشف عن اضداد الكارديولبين نوع IgM،ونوع IgG واضداد β 2GP1 نوع IgG باستخدام تقنية الاليزا .

النتائج: كان فحص أضداد الكارديولبين نوع IgM موجبًا لدى سبعة مرضى وكان المستوى مرتفعا لدى واحدا منهم فقط ، اما بالنسبة لأضداد كارديولبين نوع IgG فقد كان موجبا وبمستوى ايجابي منخفض لدى٦ مرضى وكان فحص أضداد β 2GP 1 نوع IgG موجبا لدى ٤ من المرضى فقط. لم تظهر الدراسة فروقات معنوية في مستوى أضداد الكارديولبين بنوعيها IgM و IgGوكذلك أضداد β 2GP 1 نوع IgG في مرضى السكري من الذين لديهم مضاعفات المرض عن المجموعة الخالية من المضاعفات.

الاستنتاجات:

١. كان مستوى أضداد الكارديولبين نوع IgM و IgG وأضداد β 2GP 1 نوع IgG اعلى لدى مرضى السكري من النوع ٢ عما هو عليه في مجموعة الغير مصابين بالسكري.
٢. يمكن أن يشير الفحص الموجب ولكن التركيز المنخفض لأضداد الكارديولبين وأضداد β 2GP 1 لدى مرضى السكري الى ان هذه الأضداد قد يكون لها دورا ضئيلا في حدوث تصلب الشرايين .
٣. وجود العيار المنخفض من أضداد الكارديولبين نوع IgM ونوع IgG وأضداد β 2GP 1 نوع IgG في كل من مجموعتي مرضى السكري المصحوب بمضاعفات المرض وغير المصحوب بالمضاعفات ما من شانه أن يقلل من أهمية هذه الأضداد كأحد المسببات في الاعتلال الوعائي لدى مرضى السكري.

الكلمات المفتاحية: السكري ، اضداد الشحوم الفوسفاتية ، اضداد الكارديولبين ، الاعتلال الوعائي .

INTRODUCTION

Diabetes is considered as a distinct risk factor for the development of atherosclerosis and its complications. The pathophysiological process is still not fully understood[1].

Vascular damage and endothelial cell dysfunction start early in the course of diabetic vasculopathy. Accumulated evidences demonstrated that hyperglycemia initiates certain biochemical events that lead to vascular dysfunction and subsequent structural changes in the vessels[2].

Humoral factors that may be associated with accelerated atherosclerosis are anticardiolipin antibodies and antibodies against negatively charged phospholipids, in addition to antibodies against oxidatively modified low-density lipoproteins and circulating immune complexes[3].

Antiphospholipid antibodies(aPL) are a heterogeneous family of autoantibodies acting against different phospholipids or phospholipid-binding proteins present on cellular membranes. Included in this family are antibodies to cardiolipin (aCL), antibodies to β2 glycoprotein (β 2GP 1) and lupus anticoagulant[4].

Anticardiolipin antibodies are a subgroup of aPL antibodies and they are of IgM, IgG and IgA isotypes[5].

β2 glycoprotein 1 was recognized as an adhesion molecule which may bind to phospholipids and acts as a major antigen of antiphospholipid antibodies[6]. It is a protein which might have a critical role in thrombosis with anticoagulant function. It is one of the components of the protein part of many lipoproteins such as a very low density lipoprotein[7].

Clinically, anti-human β2 GP 1 autoantibodies may be found in association with anticardiolipin antibody[8].

Because phospholipids are an integral part of platelets and endothelial cell surface membrane, it is expected that antiphospholipid antibodies would have a significant effect on platelets and vascular endothelial mechanisms[9].

The implication of aCL antibodies with vascular complications of diabetes is still argumentative[10],[11].

Many studies showed controversy about the occurrence of antiphospholipid antibodies in type 1 and type 2 diabetic patients.

We studied the presence of IgM, IgG anticardiolipin antibodies and IgG antibodies to β 2 GP1 in a group of patients with type 2 diabetes mellitus.

We aim to investigate the presence of antibodies to phospholipid and the phospholipid associated proteins in type 2 diabetic patients and to verify their possible implication in the development of thrombotic events in such patients

PATIENTS AND METHODS

The study was conducted from March 2013 to December 2013. It included a group of 50 patients with type 2 diabetes mellitus attending Diabetic Clinic in Al-Salam Teaching Hospital. They were 9 males and 41 females. Their ages range between 40 and 64 years with a mean age of 51.72± 9.26 years. These patients were of different duration of disease. Criteria for exclusion were co-existent autoimmune disorders. Vascular complications were assessed by the presence or absence of retinopathy, nephropathy and polyneuropathy.

The comparison group was included 33age and gender-matched healthy people. They were 6 males and 27 females. They have age range of 36-58 years and a mean age of 47.30±11.16 years.

All sera of both patients and comparison groups were tested for the presence of anticardiolipin IgM and IgG isotypes and for β2GP1 IgG using ELISA technique. The titer of aCL was measured in MPL and GPL international units. Positivity of aCL was defined as a titer of IgM aCL higher than 15 MPL and a titer of IgG aCL higher than 10 GPL. A titer higher than 20 SGU was considered positive for β2GP1 IgG according to the recommendation of the kits's manufacturers (IMMUNOSPEC Corporation).

Statistical Analysis

Standard statistical methods were used to estimate the mean and standard deviation. Paired t-test and two sample t-test were used for comparing the results of various parameters among the studied groups.

F- Test was used for equal variance estimation when appropriate. Some values expressed as Mean±SD and p value of <0.05 was considered statistically significant. The statistical tests were conducted by usage of *SPSS* version 19 and MedCalc version 13.1.

RESULTS

Fifty patients with type 2 DM, (9 males, 41 females) and 33 non-diabetic subjects (4 males, 29 females) were enrolled in this study.

The clinical characteristics of patients and controls are shown in Table 1.

As shown in Table 2, only one diabetic patient (2%) had a moderately elevated titer of IgM aCL, while 6 patients (12%) had low positive titers of IgM aCL. Six patients (12%) had low positive titers of IgG aCL (Table 2).

Two patients (4%) had highly positive titers of IgG β2GP1 and 1 patient (2%) had moderately elevated titer of IgG β2GP1 antibodies while the low positive titer of IgG β2GP I antibodies was found in 1 patient (2%)Tables 3, 4 and 5 showed the presence of aCL of IgM and IgG types with IgG β2GP1 antibodies among type 2 DM patients was statistically significant (p<0.0001).

Tables 6, 7 and 8 demonstrated the statistically significant higher levels of titers of IgM aCL titer, IgG aCL titer and IgG β2GP1antibodies among type 2 DM patients in correlation to the non-diabetic group.

There was no difference in the means of IgM aCL titer (18.2000), IgG aCL titer (12.3000) and IgG β2GP1 (47.5000) in patients with complicated and that of uncomplicated DM (23.4667) (12.2500) (39.8500) respectively.

Table 1: Patients and non-diabetic group haracteristics.

Variable	Pateints(DM)	Non-diabetic group
	Mean± SD, No. (%)	Mean± SD, No. (%)
Age	51.72±9.26	47.30±11.16
FBS	220.24±76.62	86.64±12.95
HbA1C	7.88±1.41	----------
Gender female	41(82)	29(87.8)
Complicated DM	32 (64)	
Retinopathy	11 (22)	
Nephropathy	16 (32)	
Cardiovascular disease	20 (40)	
Cerebrovascular disease	9 (18)	
Symptomatic peripheral Neuropathy	5 (10)	
Hypertension	37 (74)	
Hyperlipidemia	38 (76)	
Obesity(BMI>30 Kg/m2)	39 (78)	21(63.6)
Insulin therapy	19 (38)	

Table 2: Distribution of Anticariolipin and Anti- β 2 Glycoprotein 1 antibodies in diabetic group.

Anti β2GP1 antibody (IgG) No. (%)	Anticardiolipin antibody (IgG) No. (%)	Anticardiolipin Antibody (IgM) No. (%)	Titer of Antibody
2 (4%)	--------	--------	High Titer
1 (2%)	--------	1 (2%)	Moderate Titer
1 (2%)	6 (12%)	6 (12%)	Low Titer
4 (8%)	6 (12%)	7 (14%)	Total (%)

Table 3: Anticardiolipin antibodies(IgM) in diabetic patients.

Disease	Anticardiolipin Antibody(IgM)	No. (%)	P-value
DM	POSITIVE	7(14)	<0.0001
	NEGATIVE	43(86)	
	Total no. (%)	50(100)	

Table 4: Anticardiolipin antibodies (IgG) in diabetic patients**.**

Disease	Anticardiolipin Antibody(IgG)	NO. (%)	P- value
DM	POSITIVE	6(12)	<0.0001
	NEGATIVE	44(88)	
	Total no. (%)	50(100)	

Table 5: Anti- β 2 Glycoprotein 1 (IgG) in diabetic patients

Disease	Anti-β 2 Glycoprotein I (IgG)	No. (%)	P- value
DM	POSITIVE	4(8)	P <0.0001
	NEGATIVE	46(92)	
	Total no. (%)	50(100)	

Table 6: Anticardiolipin antibodies IgG (GPL/ml) level among studied groups

Studied groups	No.	Mean	SD	Std. error	Variance	P value
DM	50	4.5120	3.0259	0.4279	9.1562	
Non-DM	33	2.4152	1.3449	0.2341	1.8088	P< 0.001

Table 7: Anticardiolipin antibodies IgM (MPL/ml) level among studied groups

Studied groups	No.	Mean	SD	Std. error	Variance	P value
DM	50	4.2640	7.0802	1.0013	50.1297	
Non-DM	33	2.4152	4.3904	0.7643	19.2753	P =0.005

Table 8: Antiβ2 Glycoprotein 1 (SGU/ml) level among studied groups

Studied groups	No.	Mean	SD	Std. error	Variance	P-value
DM	50	10.6182	25.4744	3.6026	648.9470	P <0.001
Non-DM	33	9.5100	7.1355	1.2421	50.9153	

DISCUSSION

This study showed that the presence of moderate to high aCL antibody titers and β 2 GP I antibodies in patients with type 2 DM was infrequent. Similar studies such as that of *Tarkun et al* found that no case had an IgG aCL titer more than 20 GPL units[12]. *Palomo et al* showed that only four out of 100 patients had moderate level of aCL[13], the same findings were detected by *Calvo-Romero* who found low aCL titers in diabetic patients[14].

On the contrary, *Galtier et al* demonstrated that an IgG aCL titer more than 15 GPL Units was found in 9.5% of cases[11].

Our findings of frequent low positive titers of aCL antibodies could suggest that these autoantibodies may be blameless in the pathogenesis of diabetic atherosclerosis.

Our patients were mostly of old age, and the frequency of low aCL titers might be explained by the immunosenescence theory which suggest that immune dysfunction with aging can lead to increase autoantibody production[15]. This is in accordance with the study by *Fields et al*, whereby IgG and IgM aCL were detected in 12% of the healthy elderly and in 2% of younger adults[16].

On the contrary, another study showed that aCL positive results in the elderly were reported to be insignificant and similar to younger populations[17].

It was found that the presence of β 2GP1 is a crucial requirement for antibody-phospholipid interaction, indicating that bound β 2GP1 forms the antigen to which aPL antibodies are directed. However the frequency and pathogenic role of these antibodies in atherosclerosis have been a matter of discussion[18].

Although there was significant differences in the titers of IgM, IgG aCL and IgG β 2GP1 antibodies in the diabetic group compared to the comparison group, it seemed to be of no clinical importance as the mean titers of both of them is considered relatively low.

Low titers of aCL and β2 GP1 antibodies were seen in both complicated and non-complicated diabetic patients. These results, although limited by the small sample, do not favor a pathogenic effect of aCL and β2GPI antibodies in type 2 diabetic macrovasculopathy. Our data are established by those reported by *Tarkun et al.*, which recede aCL and β2 GP1 antibodies from vascular complications of type 2 diabetes[12], and also by *Copetti* who concluded that anticardiolipin antibodies do not mediate macrovascular complication of type 2 diabetes and that aCL positivity rates were similar in diabetic patients with and without vasculopathy[10].

CONCLUSION

1.The aCL and β2-glycoprotein-I antibodies positive titer means among type 2 diabetics are significantly higher than the non-diabetics.

2.Positive but low titers of aCL and β2 GPI antibodies could suggest that these autoantibodies may play a minor role in the pathogenesis of atherosclerosis.

3.Low titers of aCL and β2 GP1 antibodies were seen in both complicated and non-complicated diabetic populations that probably lessen the importance of these autoantibodies as effective contributors in the pathogenesis of diabetic vasculopathy.

RECOMMENDATIONS

Prospective studies of large populations with follow up for complications are essential to reveal this association further.

Inclusion of IgA aCL in the future studies to be tested alongside the currently studied antibodies has to be considered.

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