Antiphospholipids antibodies in type II DM patients

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المضادات المناعية للدهون المفسفرة لمرضى النوع الثاني من مرض السكري

الخلاصة

ألهدف من الدراسة: الكشف عن وجود المضادات المناعية للدهون المفسفرة لمرضى السكري النوع الثاني والبحث عن وجود علاقة احصائية بين هذه المضادات والمضاعفات الخثرية لهؤلاء المرضى.

المرضى والطرق: خلال فترة خمسة اشهر، اربع وثمانون مريض من النوع الثاني لمرض السكري تم دراستهم اثناء مراجعتهم لعيادة الطب الباطني في كربلاء- العراق. اثنا واربعون متبرع بمعدل اعمار ٥٦ سنة استعملو كمجموعة السيطرة الاصحاء. تم سحب الدم الوريدي من جميع المرضى ومجموعة السيطرة الاصحاء وتم فحص ال(LA) بالطرق الخثرية وال(aCL IgM, IgG) بالطرق المناعية.

النتائج: من مجموع اربع وثمانون مريض لمرض السكري، وجد تسع وخمسون انثى وخمس وعشرون ذكر وبمعدل اعمار كانت اثنان وخمسون سنة.حالتان فقط من المرضى وجدت موجبة لل(LA) بالمقارنة لا توجد اي حالة موجبة لمجموعة السيطرة الاصحاء كما وجدت حالة واحدة من المرضى موجبة لل (aCL IgM and IgG) وحالة واحدة اخرى موجبة لل(aCL IgG) بالمقارنة لحالة واحدة موجبة لل(aCL IgG) لمجموعة السيطرة الاصحاء. بينت المعلومات المستسقاة من المرضى ومجموعة السيطرة الاصحاء عدم وجود العلاقة الاحصائية للمضادات المناعية للدهون المفسفرة بين المرضى ومجموعة السيطرة بالاضافة الى عدم وجود مثل هذه العلاقة بين المرضى المصابين بالمضاعفات الخثرية من الذين لايملكون هذه المضاعفات.

الاستنتاج: ايجابية وجود المضادات المناعية للدهون المفسفرة لمرضى السكري هي حالة استثنائية ولاتملك اي دور في المضاعفات الخثرية للمرضى. دراسات اخرى مع توسيع اعداد المرضى ومتابعة دقيقة قد تحدد احتمالية وجود دور لهذه المضادات مع المضاعفات الخثرية في الاوعية الدموية الدقيقة والكبيرة.

Abstract

The Aims of the present study are to determine the presence of antiphospholipid antibodies (Lupus anticoagulant, IgM and IgG anticardiolipin antibodies) in a series of patients with type II diabetes mellitus and to assess any correlation between cardiovascular complication of diabetes mellitus and presence of antiphospholipid antibodies.

Patients and methods: A total number of 84 patients who were known case of type II diabetes mellitus were studied during a period of 5 months extended from June 2009 to October 2009 while attending the out patient clinic in Karbala, Iraq**.** Forty two healthy volunteers of 56 years mean age served as a control group. Blood were aspirated from all patients and control group for lupus anticoagulant (Kaolin clotting time / Kaolin clotting time index) and anticardiolipin (IgM and IgG) antibodies estimation.

Result: Total number of 84 studied patients with type II diabetes mellitus, 59 were females and 25 were males with mean age of 52 ± 4 years. Two diabetic patients have lupus anticoagulant positive result; in contrast no case in control healthy group had lupus anticoagulant positive result. One case of diabetics had positive anticardiolipin IgM and IgG antibody, in addition to another patient had anticardiolipin IgG antibody in contrast to single case of control group with IgG anticardiolipin positive result. The data obtained from those 84 cases showed no statistically significant parameters of antiphospholipid antibodies (Lupus anticoagulant, IgM and IgG anticardiolipin antibodies) between the control & patients groups and there was no significant correlation between cardiovascular complicated and uncomplicated groups regarding antiphospholipid antibodies.

Conclusions: Presence of antiphospholipid antibodies is exceptional in patients with type II diabetes mellitus, and has no role in the pathogenesis of diabetic thrombotic complications.

Further studies with high series of cases and follow-up should be elucidating the presence of antiphospholipid antibodies in type II diabetes mellitus and its macro and microvascular complications.

Introduction:

Antiphospholipid antibodies (APAs) are a family of antibodies reactive with proteins, which themselves complexed with negatively charged phospholipids [(1)]. Beta 2-glycoprotein I (b2-GP-I), a phospholipid-binding plasma protein with weak anticoagulant activity, for binding to acidic phospholipids. The precise relationships among b2-GP-I, phospholipid and autoantibody are disputed. One hypothesis postulates a defect in cellular apoptosis, which exposes membrane phospholipids to the binding of various plasma proteins, such as beta-2 glycoprotein I. Once bound, a phospholipid-protein complex is formed and a new epitope is uncovered, which subsequently becomes the target of autoantibodies [(2)]. However, other proteins share this property of binding to phospholipid in a manner that promotes interaction with antiphospholipid antibodies. These include prothrombin, annexin V, protein C, protein S, thrombomodulin and high molecular weight kininogen. Despite this improved understanding of the true nature of APAs and because the clinical utility of the newer assays is incompletely evaluated, the laboratory diagnosis of antiphospholipid syndrome still relies predominantly on coagulation-based assays for lupus anticoagulant (LA) and solid phase assays (ELISAs) using cardiolipin [(3)]. Anticardiolipin antibodies are a subgroup of antiphospholipid antibodies, and the IgG and IgM isotypes are the most important [(4)]. Antiphospholipid syndrome (APS) may be diagnosed when arterial or venous thrombosis, or recurrent miscarriage, occurs in a subject in whom laboratory tests for antiphospholipid antibody (anticardiolipin, lupus anticoagulant or both) are positive. Because thrombotic disease, miscarriage and transient antiphospholipid antibody positive result are all common events, persistence of the positive tests must be demonstrated and other causes and contributory factors considered, confirmed in separate blood samples collected at least 6 weeks apart [(3)]. The mechanisms underlying the prothrombotic state in APS have not been clarified. A range of pathogenetic mechanisms has been reported in subjects with APS, including protein C resistance, vascular endothelial autoimmunity and activation and impaired fibrinolytic capacity [(5).] Subjects with APS present to a wide range of clinical specialists for example, clinical haematologists, neurologists, cardiologists, rheumatologists, dermatologists and obstetricians [(3).]

The diagnosis of APS relies on the demonstration of the presence of either LA by coagulation tests or APAs by solid phase immunoassays. The latter typically use cardiolipin as the antigen (aCL assays). At present, the traditional tests remain the mainstay of laboratory investigation of APS and it is clear that both LA and solid phase-type assays must be used for the detection of APAs in certain patients, as cases with LA but no aCL and vice versa are well recognized. Reliance on just one type of assay may lead to false negative APAs assessments [(3).] Coagulation assays (lupus anticoagulant tests) represent a large number of diagnostic tests and modifications have been described and a number of commercial kits and reagents have been introduced. Despite this, criteria for the presence of LA remain unchanged including; Prolongation of a phospholipid-dependent coagulation test (e.g. activated partial thromboplastin time-APTT, Dilute Russell's viper venom time-DRVVT, Kaolin clotting time-KCT, and the tissue thromboplastin inhibition test-TTI), evidence of an inhibitor demonstrated by mixing studies and confirmation of the phospholipid-dependent nature of the inhibitor [(6).] Solid phase assays for APAs, such as the aCL ELISA test, have been gradually refined. The ELISA format allows bulk testing and the results are not affected by factor deficiency or the use of anticoagulants. The detection of aCL allows the diagnosis of APS in a subject with an appropriate clinical history, even when LA is absent. However, the aCL assay is not a substitute for the LA test nor does it confirm that LA is present because different antibodies appear to be responsible for the two activities [(7).]

Diabetic patients are considered to have a hypercoagulable state if they have laboratory abnormalities or clinical conditions that are associated with increased risk of thrombosis. Patients with DM certainly meet this criterion based on increased risk of thrombosis. Eighty percent of patients with diabetes mellitus die a thrombotic death. Seventy-five percent of these deaths are due to cardiovascular complications, and the remainder is due to cerebrovascular events and peripheral vascular complications. Vascular endothelium, the primary defense against thrombosis, is abnormal in diabetes. Endothelial abnormalities undoubtedly play a role in the enhanced activation of platelets and clotting factors seen in diabetes. Coagulation factors are elevated in diabetes with enhanced platelet aggregation and activation. Conversely, the level of the anticoagulant protein is decreased. The fibrinolytic system is relatively inhibited in diabetes [(8).] APAs have been described in patients with lupus erythematosus, recurrent abortion, ocular ischaemia, ischaemic heart disease, atherosclerosis, stroke and transient ischaemic attack, recipients of solid organ grafts and diabetics [(8, 9).] **The Aims of the present study** are to determine the presence of antiphospholipid antibodies (LA, IgM aCL and IgG aCL antibodies) in a series of patients with type II DM and to assess any correlation between cardiovascular complication of DM and presence of APAs.

Materials and Methods:

Patients: During a period of 5 months extended from June 2009 to October 2009, a total number of 84 patients who were known case of type II diabetes mellitus were studied while attending the out patient clinic in Karbala, Iraq, with no illness other than diabetes mellitus. Patient with personal or close family history of connective tissue diseases were excluded.

Following complete clinical and general physical examination, data were documented for each patient including patient name, age, sex, occupation, address, duration of diabetes mellitus, complications of DM mainly cardiovascular complication (ischemic heart disease, cerebrovascular disease and peripheral vascular disease).

Patients were classify into tow groups, forty tow patients without cardiovascular history and had no previous history of vascular events while another forty tow patients had such complication of DM.

Control group: Forty two healthy volunteers who had no personal or family history of diabetes, ages and sex matched, with mean ages of 56 years, served as a control group. In addition to a control plasma sample from healthy donor parallel with coagulation tests estimation.

Methods: From each patient and control group individual, appropriate amount of venous blood was withdrawn and divided into 2 aliquots for proper tubes including citrated tube for coagulation studies (KCT and KCT index) and plain tube for immunological investigation (aCL IgM and IgG).

Coagulation tests: These were performed on platelet poor plasma (PPP) within two hours of sampling in the same day using commercially available kits for KCT and KCT index estimation [10].

Immunological investigation: These were performed on patient serum for aCL antibodies (IgM and IgG) estimation using commercially available kits (Orgentec-Diagnostika/ Germany). IgM positive antibody consider with antibody titer more than 7.0 IU/L, while IgG positive antibody consider with antibody titer more than 10.0 IU/L.

Biostatistical analysis: The results were expressed as (mean ± standard deviation). Pooled t-test was used for the comparison of significant difference between the patients and control groups in the measured parameters. Statistical significant was defined as a P value < 0.05.

Results:

Total number of 84 studied patients with type II DM, 59 were females and 25 were males with mean age of 52 ± 4 years. Two diabetic patients have LA positive result (2.4%); in contrast no case in control healthy group has LA positive result (Table1). Only one diabetic patient without cardiovascular complications (2.3%) and one diabetic with such complications (2.3%) had LA positive result with KCT time more than 100 seconds and KCT index > 1.2 (Table2).

Single case of diabetes without cardiovascular complications had positive aCL IgG antibody (2.3%) and another patient with such complications had aCL IgM (2.3%) and IgG antibodies (2.3%) positive result in contrast to single case of control group with IgG aCL (2.3%) positive result (Table 1,2).

The data obtained from those 84 cases showed no statistically significant parameters of antiphospholipid antibodies (LA, aCL IgM and IgG) between the control & patients groups (Table 1) and there was no significant correlation between cardiovascular complicated and uncomplicated groups regarding APAs (Table 2).

Table (1): Antiphospholipid antibodies parameters in DM patients and control group

APA Parameters	DM with or without cardiovascular complications	Control group	P. value
KCT Positive Negative	2/84 (2.4 %) (mean 159.5 sec) 82/84 (97.6 %) (mean 74.5 sec)	0/42 (0%) 42/42(100%) (mean 63.1 sec)	not significant not significant
aCL IgM Positive Negative	1/84 (1%) (mean 17.3 IU/L) 83/84 (99%) (mean 3.6 IU/L)	0/42(0%) 40/42(100%) (mean 2.9 IU/L)	not significant not significant
aCL IgG Positive Negative	2/84 (2.4 %) (mean 34.6 IU/L) 82/84 (97.6 %) (mean 4.2 IU/L)	1/42 (2.3%) (mean 16.7 IU/L) 41/42 (97.9%) (mean2.9 IU/L)	not significant not significant

(P value < 0.05 considered significant)

Table (2): Antiphospholipid antibodies parameters in DM patients with and without cardiovascular complications.

APA Parameters	DM without cardiovascular complications	DM with cardiovascular complications	P. value
KCT Positive Negative	1/42 (2.3%) (mean 160 sec) 41/42 (97.7%) (mean 73.4 sec)	1/42 (2.3%)(mean 159 sec) 41/42 (97.7%) (mean 76.1 sec)	not significant not significant
aCL IgM Positive Negative	0/42 (0%) 42/42 (100%) (mean 3.2 IU/L)	1/42 (2.3%) (mean 17.3 IU/L) 41/42 (97.7%) (mean4.0IU/L)	not significant not significant
aCL IgG Positive Negative	1/42 (2.3%) (mean 34.0 IU/L) 41/42 (97.7%) (mean 2.8 IU/L)	1/42 (2.3%) (mean 35.2 IU/L) 41/42 (97.7%) (mean 5.6IU/L)	not significant not significant

(P value < 0.05 considered significant)

Discussion:

DM is systemic disease complicated by many vascular events. Antiphospholipid (APAs) antibodies have already been associated with many clinical conditions, including venous and arterial thrombosis, as well as recurrent fetal loss. In non-diabetic subjects the association between APAs and thrombosis may suggest that a causative role exists for these antibodies directed against negatively-charged phospholipids with possible pathogentic roles including protein C resistance, vascular endothelial autoimmunity and activation and impaired fibrinolytic capacity[5, 11, 13]. However, a significant association between APAs and DM has not been widely reported yet. In the present study the prevalence of APAs in diabetic patients and their significant in thrombotic complications were studied. Some studies showed that there is a significant association of thrombotic complications of DM and the presence of APAs, while others disagree with such explanation of thrombotic event in type II DM.

Our study demonstrate no statistical significant difference in the frequency of APAs between type II diabetic patient and control group, with no statistical correlation between in vivo levels of these antibodies and cardiovascular complications of such patients, and these findings were similar to other studies as Jose Maria et al [14], P Gargiulo et al [9], and T.J. Hendra' et al [11].

Jose Maria et al[14] found that the presence of moderate to high aCL titers must be exceptional in patients with type II DM and low aCL titers may occur in such patients. These low titers do not seem to be associated with complicated DM, cardiovascular disease, nephropathy or retinopathy. While study of Triolo G et al [12] showed an increased incidence of anti-phospholipid antibodies in diabetic patients compared with control subjects with the presence of significant association of anti-phosphatidylethanolamine antibody with proliferative retinopathy of DM.

These differences may be attributed to difference in the pathophysiological nature of diabetic's patients in different society, difference in the mass of studded cases, facilities used in parameters measurement in addition to requirement for detection of other antiphospholips antibody as anti-beta2 GP1.

Conclusions:

APAs positive result is exceptional in patients with type II DM, and has no role in the pathogenesis of diabetic thrombotic complications.

Further studies with high series of cases and follow-up should be elucidating the presence of APAs in type II DM and its macro and microvascular complications.

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