Research Article

The Correlation between the levels of Coxsackie B viruses Ig's and the glutamic decarboxylase auto antibodies in diabetes mellitus type 1 patients

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Abstract

About 44% of diabetes mellitus type 1 (DM1) patients were positive for one or more Coxsackie B virus IgM Serotypes, compared to zero in the healthy control subjects, those samples showed a significant strong positive correlation with glutamic acid decarboxylase auto-antibodies, this may indicate the role of these viruses in the pathogenesis of DMI.

This work was done in cooperation between Baghdad University, Baghdad – Iraq and the National Center of diabetes, endocrinology and genetic, Amman – Jordan, in a sabbatical leave study.

Keywords: Coxsackie B virus, Glutamic acid decarboxylase auto-antibodies, Type 1 diabetes mellitus

INTRODUCTION

Diabetes Mellitus type 1 (DM 1) is an autoimmune disease, in which pancreatic cells are selectively destroyed by the cells of the immune system.[1] There are four known types of the auto-antibodies for the islet antigen group, insulin or pro-insulin, glutamic acid decarboxylase (GAD), tyrosine phosphate and zinc transporter antibodies.[2, 3] The auto-antibodies for GAD, which is one of the major or Beta cells target antigens, may appear years before the clinical onset of DM1, and has been observed to vary form 62-84% among the newly diagnosed patients.[3, 4]

Enteroviral viral infections as an environmental factor were significantly more common in recently diagnosed DM1, patients.[5, 6] A high frequency of immune response for different Coxsackie B virus (CBV) serotypes was reported.[6-8] Therefore, the aim of this study was to investigate if there is any relation or correlation between the level of GAD auto antibodies and CBV antibodies for different serotypes in the newly diagnosed children with DMI.

PATIENTS AND METHODS

Subjects

Fifty serum samples were obtained from DM1, patients, 25 male, 25 female, with an age range of 6-17 years. Diagnosis, treatment and follow up evaluation were carried out by the consultant medical staff at their clinics. The healthy control group included 25 serum samples, 13 male, 12 female and they were age matched with the study group.

Solid phase ELISA for GAD – IgG auto antibodies detection

The anti GAD – IgG – autoantibodies were assed using ELISA kit assay (Euroimmun, AG, UK), which provides

a quantitative assessment for human auto-antibodies against GAD in serum. The instructions of the manufacture were followed for the procedure.

Indirect immuno fluorescence assay for CBV – 1 – 6 IgG & IgM

Antibodies of class IgG and IgM for six Coxsackie B virus serotypes (1-6) were assayed using indirect immuno fluorescence assay IF kits (Euroimmun, AG, UK), which provides quantitative and qualitative assessment for CBU antibodies. The instructions of the manufacture were followed for the procedure.

Statistical method

t test, chi-square test and correlation value were used, P value < 0.05 was considered statistically significant.

RESULTS

The mean serum level of anti – GAD auto antibodies in DM1 patients serum samples is shown in table (1) compared to the control group. A significant difference was found.

Table 1. Mean serum level of anti GAD auto antibodies in DM1 patient and control.

Sample	No.	Mean serum level ± S.E
Patients	50	55.6 ± 13.52
Control	25	4.49 ± 0.03
P-valve	< 0.05	Significant

S.E. standard deviation.

The response for CBV (1-6) IgG in the DM1 patients and the control subjects is shown in table (2), while the response for CBV (1-6) IgM in the DM1 patients and control subjects is shown in table (3).

Table 2. Anti CBV (1-6) IgG response in DM1 patients and the control subjects.

	B1 (%)	B2 (%)	B3 (%)	B_4 (%)	B5 (%)	B6 (%)
No. + ve DM_1 Samples (%)	40 (80%)	42 (84%)	46 (92%)	44 (88%)	46 (92%)	44 (88%)
No. + ve Samples Control (%)	25 (100%)	25 (100%)	23 (92%)	22 (88%)	23 (92%)	22 (88%)
P Value	> 0.05	NS *				

*NS = non significant.

Table 3. Anti CBV (1-6) IgM response in DM1 patients and the control subjects.

	B1 (%)	B2 (%)	B3 (%)	B4 (%)	B5 (%)	B6 (%)
No. + ve DM_1 Samples	8 (16%)	16 (32%)	4 (8%)	14 (28%)	20 (40%)	10 (24%)
No. + ve Control Samples	0	0	0	0	0	0
P Value	< 0.05	S *				

*S = significant.

Table (4) shows the correlation between the level of GAD auto-antibodies and the level of CBV IgG antibodies in DM1 patients, similarly table (5) shows the correlation between the level of GAD auto antibodies and the level of IgM – CBV antibodies.

Table 4. The Correlation between the level of GAD auto antibodies and the level of CBV (1-6) IgG antibodies.

	R Valve	P Valve
CB1 – GAD	0.740	S *
CB2 – GAD	0.410	NS **
CB3 – GAD	0.400	NS **
CB4 – GAD	0.200	NS **
CB5 – GAD	0.200	NS **
CB6 – GAD	0.200	NS **

*S = significant P < 0.05.

**NS = non significant p > 0.05.

R value: coefficient of correlation.

Table 5. The Correlation between the level of GAD auto-antibodies and the level of CBV (1-6) IgM antibodies.

	R Valve	P Valve
CB1 – GAD	0.22	NS **
CB2 – GAD	0.210	NS **
CB3 – GAD	0.610	NS **
CB4 – GAD	0.310	NS **
CB5 – GAD	0.930	S **
CB6 – GAD	0.920	S **

*S = significant.

**NS = non significant.

DISCUSSION

In this study anti GAD auto-antibodies showed a significant increase in serum level in the newly diagnosed patients, compared to the control group (table 1). Some studies indicated that either GAD or insulin auto-antibodies is associated with slower progression of the disease, others demonstrated a high prevalence of anti – GAD serum levels in relation to age onset also ethnic and racial differences could not be excluded.[4, 9, 10]

Coxsackie B Viruses belong to the genus of enteroviruses, which encompasses more than 60 serotypes, those viruses were reported to play an important role in the development of DM1 as an environmental factor, supported by may epidemiological and serological observations.[6, 8, 10-13] Tables (2 and 3) show the results for detection IgG and IgM for the six CBV serotypes by IF in serum of the newly diagnosed DM1 patients. The results showed non significant differences for CBV - IgG compared to the control group, that may be explained on the fact that the previous infection for CBV is common among children. On the other hand, 44% of serum samples were positive for IgM - CBV serotypes compared to zero in the control group. This difference is highly significant for all CBV serotypes. Furthermore, a single sample was positive for more than one serotype (table 3); the highest response was for IgM to CBV5, which showed 40% response among all tested samples. These results were similar to what was reported by other investigations, a 39% response to anti IgM CBV serotypes was reported,[12] others indicated 40% of tested samples were positive,[13] and 46% was reported by another study.[7] However, there was a variation in identifying which serotype is responsible for this positivity, some indicated CBV2 and CBV3,[13, 14] CBV4 was also reported,[4, 13] whatever is the serotype involved in this response, all there studies may indicate that CBV infections are involved in the initiation of the autoimmune attack on the islet beta cells.[7, 10, 11] However, such infections in children may be the case in less than half of the patients though with seasonal and annual peaks and changes serotypes may reflect the natural epidemiology of these viruses.[13] A genetic factors may be involved, that this high frequency of the positive response to CBV serotypes was found to be higher in DM, patients who express DRB* 104 MHC haplo type.[7] The hypothesis of a relationship of these viruses to the disease has been strengthened by the presence of entero viral components or infectious particles or viral RNA in the blood samples, and biopsies of DM, patients.[6, 16] Recently it was indicated that the infected beta cells produce (IFN) and this cytokine can initiate the autoimmune response toward the B cells.[11, 15, 16] The major problem in interpreting serological studies on enteroviruses is the lack of data on sensitivity, specificity and comparability of the used assays, which complicate the identification of the exact serotype due to the cross reactivity between entero- viruses, the existence of distinct viral strains which are indistinguishable on standard serotype assays and the studies on CBV – RNA detection by PCR are few.[7, 8]

Table (4, 5) represents the correlations between the levels of the GAD auto antibodies and levels of anti CBV – IgG – IgM antibodies respectively, the correlation was significant and positive with anti – IgG – CBV1 and anti – IgM – CBV 5-6. Such correlations may be explained that there is a sequence homology between 65 kD beta cell antigen glutamine acid decarboxylase and CBV P2-C protein which shares an exact 6 amino acid match.[4, 6, 17]

Finally, as a conclusion, the high percentage for CBV – IgM antibodies in serum of DM1 patients and their correlation to the GAD autoantibodies may proof the role of these viruses in the pathogenesis of DM1. To identity which serotype is responsible a molecular study is highly recommended.

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