

# **Investigation of Circulating anti-Coxsackie B, anti- Polio and anti-Adeno IgG in newly diagnosed T1DM Children**

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## **Summary:**

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**Back ground:** Viruses may be involved in the pathogenesis of Type 1 Diabetes Mellitus (T1DM), either through direct  $\beta$ -cell infection or as triggers of autoimmunity.

**Objective:** To investigate the presence of specific anti-viral IgG antibodies for Coxsackie virus type B (CVB5), Poliovirus, and Adenovirus which proposed to be involved in the etiology of T1DM.

**Subjects & methods:** A total of 60 Iraqi T1DM children were included in the presents study. They were new onset of the disease (diagnosis was from one week up to five months). For the purpose of comparisons, 50 apparently healthy control subjects were selected. Serum IgG against Coxsackie virus type B<sub>5</sub>, Adenovirus type 3, 4, and 7, and Poliovaccin Trivalent were detected quantitatively with an indirect ELISA.

**Results:** High proportion of anti-CVB5 IgG (20%)( $p < 0.05$ ) and anti- Polio IgG (31.67%) were found in T1DM children compared to controls (8%, 26% respectively), while anti- Adeno IgG were detected in diabetic patients only (6.67%)( $p < 1.0001$ ).

**Key Words:** T1DM, Anti- CVB5 IgG, Anti- Polio IgG, Anti-Adeno IgG.

## **Introduction:**

Type 1 Diabetes Mellitus (T1DM) is characterized by destruction of  $\beta$ - cells in Pancreatic islets. The process which finally leads to complete beta cell loss and onset of clinical disease starts years before any clinical symptoms and it is thought to result from several factors involving host genes, autoimmune responses and cytokines, as well as environmental factors (1).

The evidence that viral infection might cause T1DM is derived from studies where virus particles known to cause cytopathic or autoimmune damage to  $\beta$  cells have been isolated from the Pancreas (2). Several viruses have been implicated including Enteroviruses (EVs) that have been indicated to be associated with the onset of T1DM in both epidemiological, serological as well as by the studies of the viral antigen (3). A definite islet-cell tropism of EVs was demonstrated in the human Pancreas (4). The EVs of human origin include: Coxsackie viruses of group A (CVA), type 1-24 (there is no type 23); Coxsackie viruses of group B (CVB), type 1-6; Polioviruses, type 1-3; Echoviruses, type 1-33 (no type 10, 22, 23 or 28) and Enteroviruses, type (68-71) (5). Enteroviruses are transient inhabitants of the human alimentary tract and

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may be isolated from the throat or lower intestine (6). Many investigators reported a frequent occurrence of EV mRNA in serum samples taken from children at the time of diagnosis (7). A longitudinal study conducted by Buschard and Madsbad, 1984 found that CVB4 antibody titer fell from the diagnosis and the 5 months to the 2 years study in T1DM patients, and the average titer was also lower than in healthy control individuals. In contrast, another investigators were demonstrated high levels of specific IgM antibodies to CVB in most newly diagnosed T1DM children (9, 10). In Finland another study found that non of the children vaccinated against Poliomyelitis had antibodies to the diabetes associated epitope to tyrosine phosphatase IA2, but the same diabetic children had high levels of specific IgM antibodies to Poliovirus derived VP1 peptide at onset of T1DM (11).

Adenoviruses are divided into six groups (A-F) containing 41 serotypes. They commonly infect human causing acute illness, mainly of the respiratory (the common cause of colds with fever) and intestinal tract (6).

In order to gain more understanding about the role of viral infection with initiation of T1DM, T1DM Iraqi children were serologically studied for the presence of anti-CVB5, anti- Polio and anti- Adeno IgG.

### Subjects, Materials and Methods:

Sixty Iraqi T1DM children were subjected to this study. The patients were attending to National Diabetes Center at Al-Mustansiriya University/ College of Medicine during the period May 2004 to October 2005. Their ages range from 3 -17 years, and they were new onset of the disease (diagnosis was from one week up to five months). Diagnosis of Diabetes Mellitus and selection of patients was accomplished with the assistance of the consultant medical staff in the National Diabetes Center according to the criteria as listed in the report of the expert committee on the diagnosis and classification of diabetes mellitus (12). All the patients were treated with daily replacement doses of insulin at the time of blood sampling. For the purpose of comparisons, 50 apparently healthy control subjects matched for age (4-17) years old and sex were selected who have no history or clinical evidence of type 1 diabetes or any chronic diseases and obvious abnormalities as a control group.

Three milliliter of blood was collected into plain test tubes, then the serum was separated by centrifugation at 2500 rpm for 10 min., divided into aliquot and kept at -20°C until used. Three viruses were used in the present study includes Coxsackie type B<sub>5</sub> KBR-CF antigen (Virion, France), Adeno KBR-CF antigen type 3, 4, and 7 (Virion, France), and Polioral Trivalent, Chiron, S.P.A. (WHO, Aventis). Standardization was carried out for the three tested viral antigens, antisera includes positive and negative human CVB<sub>5</sub> sera, positive and negative human aAdenovirus sera (Virion, France), positive human Poliovirus sera obtained from vaccinated children, negative human Poliovirus sera obtained from old person (more than 60 years old), and anti-human IgG with Horse-Reddish Peroxidase (HRP) conjugate, (Sigma, Germany).

Pooled positive human oliovirus sera was obtained from 10 individual samples of vaccinated children (5 vac<sup>+</sup>) with a period not less than 3-4 weeks from the last vaccine given to the child. The pool antisera was then divided into small amount and stored at -20°C (13). Checker board test was done for the determination of optimal concentration of antigens, optimal antisera dilution and optimal anti-human conjugate dilution.

The following concentrations and dilutions were specified by standardization procedure:

- Optimal concentration for CVB<sub>5</sub> antigens = 50 µg/ml.
- For Adenovirus 3, 4, 7 antigens = 12.5 µg/ml.
- For oral Poliovirus vaccine concentration = 1:2.
- Optimal serum dilution for CVB<sub>5</sub>, Adenovirus and oral Poliovirus vaccine =1:2.
- Optimal anti-human IgG conjugate dilution = 1/1000.

Serum IgG against CVB<sub>5</sub>, Polio and Adenovirus antigens can be detected quantitatively with an indirect ELISA as described by (14, 11). The microtiter plates were coated by 50 µl of each of the tested viral antigen solution in carbonate buffer pH 9.6. The plates were incubated overnight at 4°C, washed twice with phosphate-buffered saline (PBS) supplemented with 0.1% Tween-20,

then blocked with 275 µl 0.1% bovine serum albumin (BSA) in PBS to prevent non-specific binding at room temperature for 30 min. The plates were washed 3 times with PBS supplemented with 0.1% Tween-20 and were then incubated with 50 µl of human serum (1:2 dilution) in incubation buffer (PBS supplemented with 0.1% Tween and 1% BSA) at 37°C for 1 or 2 hrs. The plates were washed as above, and antibodies were detected by peroxidase- conjugated antihuman IgG at a dilution of 1:1000. Cutoff value at each run was calculated by getting the mean of OD reading for the 8 wells that contains negative anti-viral antibodies (control negative) plus 2 standard deviation (SD). Sample value lie below the cutoff value (mean negative + 2 SD) were considered negative. Those who were equal or greater than cutoff value were considered positive (15).

Statistical analysis was performed by using Chi Square test.

**Results:**

**Serological Finding for Anti-Viral IgG Anti-CVB<sub>5</sub> IgG in T1DM Patients:**

The value which is equal or higher than cutoff OD value (0.138) considered as sero positive. Only 12 patients out of 60 were sero-positive (20%) compared to 4 healthy individuals out of 50 (8%) who were sero-positive for anti-CVB<sub>5</sub> IgG. These differences were statistically significant (P=0.048) (table -1).

**Table-1: Prevalence of sero positive / negative IgG against CVB<sub>5</sub> in control and T1DM patient groups.**

Virus	Groups	No.	Sero positive		Sero negative		P <sub>1</sub>
			No.	%	No.	%	
CVB <sub>5</sub>	Controls	50	4	8.0	46	92.0	0.048 (S)
	T1DM	60	12	20.0	48	80.	

Chi= 2.994

**Anti-Polio IgG**

As shown in table (2), out of 60 patients 19 (31.67%) were sero-positive for anti-Polio-IgG compared to 13 (26%) healthy

controls, so no difference appeared between both groups (P = 0.649). The value which is equal or more than cutoff OD value (0.178) is considered as sero positiv

**Table -2: Prevalence of sero positive / negative IgG against Poliovirus in control and T1DM patient groups**

Virus	Groups	No.	Sero positive		Sero negative		P <sub>1</sub>
			No.	%	No.	%	
Polio	Controls	50	13	26.0	37	74.00	0.649 (NS)
	T1DM	60	19	31.67	41	68.33	

Chi = 0.207

**Anti-Adeno IgG**

As shown in table (3), only 4 patients were sero positive for anti-Adeno IgG (6.67%) compared with the control group who were all sero-negative. The values which were equal or

higher than 0.2 cutoff (OD) value are considered as sero-positive. This differences were highly significant between the two groups (P= 0.000).

**Table -3: Prevalence of sero positive / negative IgG against Adenovirus in control and T1DM patients groups**

Virus	Groups	No.	Sero positive		Sero negative		P <sub>1</sub>
			No.	%	No.	%	
Adeno	Controls	50	0	0	50	100.00	0.000 (HS)
	T1DM	60	4	6.67	56	93.33	

**Discussion:**

Viral involvement has long been suggested in the etiology of T1DM. The present results described finding of IgG antibodies against CVB<sub>5</sub> to be more frequent (20%) in T1DM patients than in controls (8%). A low prevalence of specific CVB-IgG may be due to use only one CVB serotype (CVB<sub>5</sub>) and there may be another CVB serotype in the sera of T1DM patients which is not detected. The frequency of IgG antibodies against Poliovirus (Oral sabin) was more (31.67%) in diabetic patients than in controls (26%). Also IgG antibodies against Adenovirus were detected in only four diabetic children (6.67%).

The presence of CVB<sub>5</sub>, Poliovirus and Adenovirus specific IgG antibodies may be evidence of previous infection in T1DM children.

The low prevalence of anti-Polio-IgG determined in healthy children may indicate a failure of Poliovaccine to enhance the immune system, although these children presumably had taken many boosted doses of oral Poliovaccine.

Several studies have found CVB-specific IgM antibodies to be more common in newly diagnosed children compared to healthy individuals (9, 10). Others detected an increase of anti-Enterovirus antibody levels (both IgM and IgG) preceding the appearance of signs of autoimmunity reflected either by synthesis of several autoantibodies or the development of clinical disease (16). However, not all studies seeking association between the Enterovirus infections (determined by increase in anti-EV antibodies)

have reported positive results. No evidence of increased antibody frequencies against CVB1, 2, 3, 4, 5, and 6 serotypes was found at the onset of childhood diabetes (17, 18). Lower antibody titer against CVB3-5 serotypes and Adenovirus 7 were demonstrated in newly diagnosed T1DM children than in healthy controls (8).

These discrepancies could be due to the fact that in all these studies, the determination of viral infection was carried out indirectly through the determination of anti-viral antibodies and it is noteworthy that studies used multiple approaches to identify these viruses (serology, PCR, Faeces analysis) appear more likely to report an association with T1DM or islet autoimmunity, suggesting that the sensitivity of viral detection is an important factor.

Enteroviruses could be involved in the pathogenesis of T1DM by several different mechanisms: During infection, viruses may reach the pancreatic islet and destroy insulin-producing β-cells by virus-induced cytolysis (19). Alternatively, β-cell damage might result from virus-induced inflammatory reactions through producing inflammatory cytokines (IL-1β, IFN-α... etc.) (20). In addition β-cell destruction might be based on molecular mimicry, because immunological cross-reactions between Enteroviruses and β cell autoantigens (GAD-65, Tyrosin phosphatase IAR/IA3) can take place at least *in vitro* (11).

**Recommendations:**

Using molecular techniques (PCR) to identify the viral infection in addition to



serological methods which appear more likely to report an association with T1DM.

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