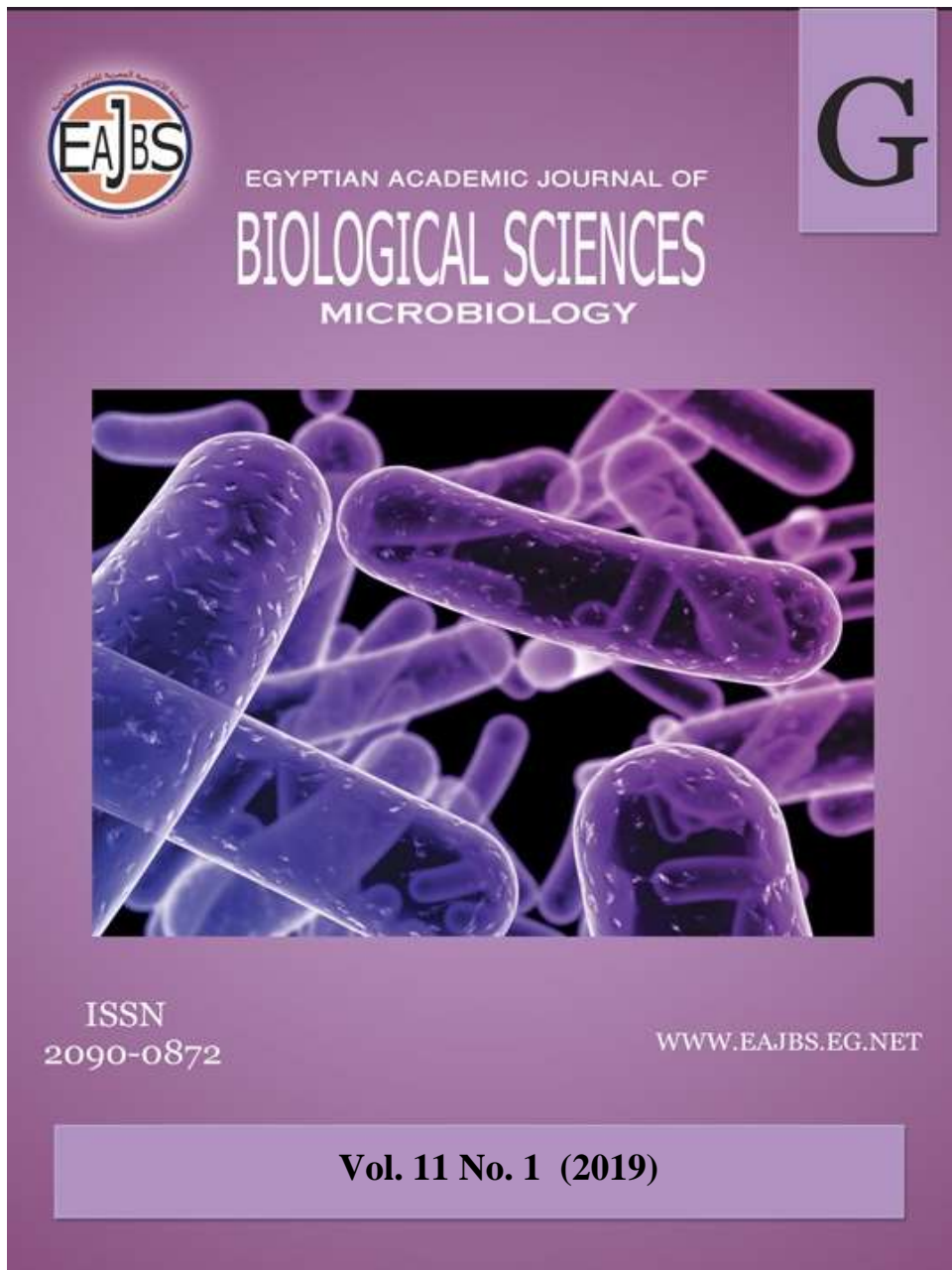


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Relation of Coxsackie B3 and B4 Viral Infections for Development of type 1 Diabetes Mellitus in Children: A Case-Control Study

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ABSTRACT

Background: Human Type 1 diabetes (T1D), previously called Juvenile-onset diabetes is one of the most common chronic, multifactorial diseases of autoimmune origin with a strong genetic component, affecting about 542 000 children in the world and represents about 5-10% of all cases of diabetes. Human enteroviruses (HEVs), particularly Coxsackie B viruses (CVBs), might trigger the onset of type 1 diabetes (T1D).

Objectives: Find out any relation between the Coxsackie virus type B3 & B4 infections in addition to GAD65 autoantibodies and the development of T1DM

Patients and Methods: A matched case-control study was conducted and sixty cases and 120 controls were enrolled in the study. Enzyme-Linked Immunoassay (ELIZA) technique was used to detect IgM and IgG in serum against the Coxsackie B3, B4 and GAD65 (Glutamic Acid Decarboxylase 65) autoantibodies of both cases and controls. Qualitative detection of the RNA of the Coxsackie B3 & B4 viruses in the cases and controls by the conventional PCR method using suitable primers in both cases and control. Molecular detection of the CB3 and CB4 RNA was done using according to the manufacturers' instruction.

Results: The following risk factors were found to be independently associated with illness, it were significantly associated with illness and at higher risk of T1DM: CB4 IgM Positivity (OR 47 [95% = 6.1-364.1], p = 0.0002), CB4 RNA Positive (OR 39.6 [95% CI = 5.1 - 309], p = 0.0004) IgG Antibodies against both CVB3 and GAD65 (OR 32.9 [95% CI = 4.2 - 258.7], p = 0.0009), GAD65 IgG Positivity (OR 11.8 [95% CI = 4.4 - 31.2], p = 0.001) and IgM Antibodies against both CB4 and GAD65 (OR 8.8 [95% CI = 2.7 - 28.2], p = 0.0002).

Other risk factors like CB3 IgM Positivity (OR 1.7 [95% CI = 0.6 - 4.5] p = 0.2), CB3 IgG Positivity (OR 1.3 [95% CI = 0.7-2.4], p = 0.3), CB4 IgG Positivity (OR 2.1 [95% CI = 0.9-4.4], p = 0.06) and IgM -Antibodies against both CB3 and GAD65 (OR 1.3 [95% CI = 0.3 - 5.0], p = 0.6) with a moderately increased risk of illness, but these were not statistically significant.

CB3 RNA Positive (OR 0.8 [95% CI = 0.3 - 1.9] p = 0.6), GAD65 IgM Positivity (OR 0.7 [95% CI = 0.4 - 1.5], p = 0.7), IgG Antibodies against both CB4-GAD65 (OR 0.9 [95% CI = 2.9 - 8.8], p = 0.05) was not associated with illness as these were not statistically significant with the decreased risk of illness and has protective role against infection with T1D.

Conclusions: We propose that children aged less than 17 years are at risk of T1D infection if exposure to CB4 whereas CB3 has protective role.

INTRODUCTION

Human Type 1 Diabetes Mellitus (T1DM), previously called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes is one of the most common chronic multifactorial diseases of autoimmune origin with a strong genetic component (Christoffersson *et al.*, 2016).

According to the World Health Organization, 180 million individuals are living with diabetes and approximately 5-10% (18 million) have T1DM (Jensen *et al.*, 2011). The disease is caused by selective destruction of the insulin-producing β cells in the islets of Langerhans of the pancreas resulting in a gradual loss of insulin production leads to the hyperglycemia and if uncontrolled, to the life-threatening state of ketoacidosis. T1DM is considered a childhood disease because most patients develop T1DM by less than 20 years of age and more often affecting children less than five years of age (Christoffersson *et al.*, 2016; Ziegler *et al.*, 2013; Karvonen *et al.*, 2000).

The true cause of type 1 diabetes is unknown, but both genetic predisposition and environmental factors are thought to affect both the initiation and the rate of T1DM disease progression (Atkinson *et al.*, 2014; Pociot and Lernmark 2016). Genetics is an important factor in the development and predilection of type 1 diabetes, but environmental factors are the triggers for the disease (Pociot and Lernmark 2016).

However, since only a small fraction of people with this genetic risk eventually develop the T1DM; the environmental factors are believed to play the important role in the pathogenesis of the disease. Type 1 Diabetes Mellitus is thought to be triggered by many factors, as certain viruses, some dietary factors such as cow's milk protein, neonatal delivery, antibiotics and host microbiome (Insel *et al.*, 2015; Kostic *et al.*, 2015).

Human enteroviruses (HEVs), particularly the Coxsackie B viruses (CVBs) was thought to trigger the onset of T1DM either by direct infection of the insulin-

producing beta-cells or by an indirect inflammatory response (Atkinson *et al.*, 2014).

Literature shows the involvement of enteroviruses in development and/or accelerating of type 1 diabetes mellitus (T1D) (Coppieters *et al.*, 2012). Recently, a high-frequency immune response for different coxsackie B virus (enteroviruses) serotypes were reported among newly diagnosed T1D (Hober *et al.*, 2013).

Reports from Iraq showed increase in the prevalence of T1D (Almahhfooth *et al.*, 2017). It was explained by changing economy in Iraq. This reported increase might be out of enhancement of transmission of enteroviruses e.g. coxsackievirus. It is a result of social strife (widespread violence and internal displacement of families).

In Iraq, there is no previous work that studied the relation of the Coxsackie virus types with the development of T1DM. The aim of the study was to show an association between the Coxsackie B3 & B4 virus infections and the development of T1DM in patients less than 17 years age group.

Hepatocellular carcinoma (HCC) is the most widely recognized primary liver tumor (Balogh *et al.*, 2016; Abdel-Hamid *et al.*, 2018). Incidence varies widely between geographical areas, probably because of variations in the exposure to hepatitis virus and other environmental pathogens (El-Serag, 2001). The clinical risk factors include cirrhosis, chronic

MATERIALS & METHODS

This is a case-control study applied in the Children's Central Hospital in Baghdad and in the Microbiology Department of the College of Medicine, Al-Anbar University during the period from the 30th of January to the 30th of September 2018 for investigating of a group of recently diagnosed T1DM aged less than 17 years admitted to the hospital and compare each case with two (2/1) age, sex and residency matched healthy controls selected at the same time of diagnosis of the index case

from the health centers and kindergartens in Baghdad to find out any relation between the Coxsackie virus type B3 & B4 infections and the development of T1DM. A total of 60 newly diagnosed T1D patients and 120 healthy controls were included in this study. The sample was age, sex and residency matched controls. Enzyme-Linked Immunoassay (ELISA) technique was used to detect IgM and IgG in serum against the Coxsackie B3, B4 and GAD65 (Glutamic Acid Decarboxylase 65) autoantibodies of both cases and controls. Qualitative detection of the RNA of the Coxsackie B3 & B4 viruses in the cases and controls by the conventional PCR method using suitable primers in both cases and control. Molecular detection of the CB3 and CB4 RNA was done using according to the manufacturers' instruction.

Five ml of blood was collected from both cases and controls. Two ml immediately centrifuged at 3500 RPM at room temperature and stored at -20°C for use for ELISA for testing for the IgM & IgG antibodies levels. The other 3 ml were collected in a sterile EDTA containing tubes, centrifuged and plasma separated and stored at -80°C until. All the EDTA blood samples were rapidly aliquoted in 100 μl , mixed with RNase inhibitor (Boehringer, Mannheim, Germany), 20 IU for every 100 μl for molecular study.

Statistical comparison of the IgM &

IgG antibodies and PCR RNA results between the cases and controls to assess the risk significance of the Coxsackie B3, B4 and GAD65 infection for the development of T1DM in addition to GAD65 autoantibodies using the odds ratio by epi-info and SPSS Version 23. OR and 95%CI was done to assess the differences between cases and control in antibodies against CVB3 and CVB4, autoantibodies against GAD65 and RNA. P value < 0.05 was considered significant.

RESULTS

The age of T1D patients was 8.3 ± 4.1 year and the age of healthy control was 9.7 ± 4.7 year. No significant difference in age was noticed between cases of T1D and healthy control ($t = 1.5$, d.f. = 178, $p = 0.1$). The male to female ratio was 1.3:1 in cases and control.

Serologic testing for the diagnosis of CB3 and CB4 viruses in addition to GAD 65 autoantibodies infection involves measurement of a panel of distinct specific antigens and host antibodies that react to these antigens.

The results of CVB3 RNA on the agarose gel electrophoresis revealed the presence of a small DNA band with a molecular weight of about 234 base pair fragment was amplified, which confirmed the RNA genome of CB3 virus in total RNA extracted from the plasma of T1DM patients (Fig. 1).

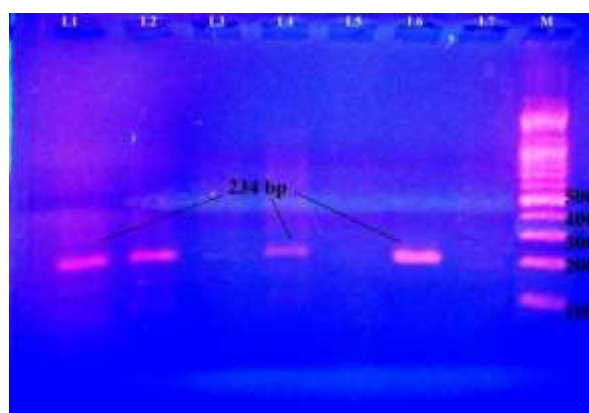


Fig. 1: Analysis of RT-PCR product using 1% agarose gel electrophoresis. The amplified 234 bp fragment confirmed the RNA genome of CVB3 in the extracted total RNA. Lane (M) Molecular weight markers (100 bp DNA ladder). Lane (1) Positive control. Lane (3) Negative control. Lane (5) Negative sample. Lanes (2, 4 & 6) positive samples resulting from PCR product.

The results of CVB4 RNA on the agarose gel electrophoresis revealed the presence of a small DNA band with a molecular weight of about 436 base pair fragment after second

PCR amplification was amplified which confirmed the RNA genome of CB4 virus in total RNA extracted from the plasma of T1DM patients (Fig. 2).

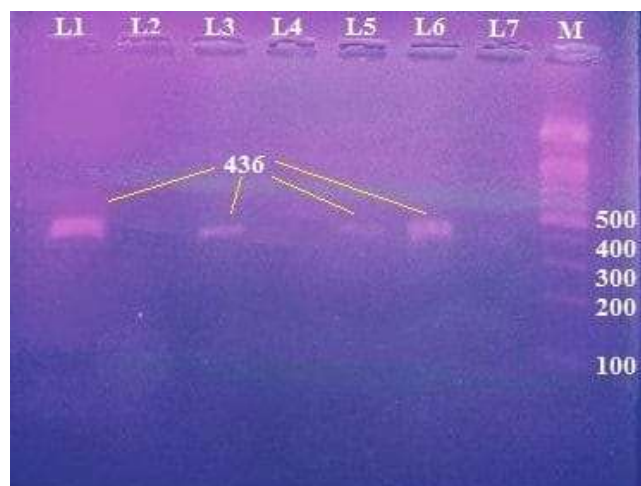


Fig. 2: Agarose gel electrophoresis revelation of semi-nested RT-PCR products. Lane(M) Molecular weight markers (100 bp DNA ladder). Lane(1) Positive control. Lane (2) Negative control. Lane(4) Negative sample. Lanes (3, 5 &6) positive samples resulting from second PCR product

CVB4 IgM was detected in 17 (28.3%) of the T1D patients and 1 (0.83%) of the controls. The presence of CVB4 IgM was significantly associated with T1D (OR 47 [95% = 6.1-364.1], $p = 0.0002$).

In 8 (13.3%) T1D patients and 10 (8.3%) controls, CVB3 IgM was detected. CVB3 IgM was not significantly associated with T1D (OR 1.7 [95%CI = 0.6 – 4.5] $p = 0.2$).

In T1D patients and controls CVB4 IgG was detected in 16 (26.7%) and 18 (15.0%) among T1D patients and controls, respectively. The association between CVB4 IgG and T1D was not significant (OR 2.1 [95%CI = 0.9-44], $p = 0.06$).

Out of the T1D patients and controls, 31 (51.7%) and 54 (45.0%) were positive for CVB3 IgG. No significant association between T1D and CVB3 IgM (OR 1.3 [95%CI = 0.7-2.4], $p = 0.3$).

RNA of CVB4 and CVB3 was detected in 15 (25.0%), 9 (15.0) of T1D patients and 65 and in 1 (0.83%) and 21 (17.5%) of healthy controls, respectively. CVB4 RNA was significantly associated with T1D (OR

39.6 [95%CI= 5.1 – 309], $p = 0.0004$) and CVB3 RNA was not significantly associated with T1D (OR 0.8 [95%CI= 0.3 -1.9], $p = 0.6$).

As some patients were developed two types of antibodies at the same time one for CVB and other for GAD65 and the results of those patients as follows:-

IgM Antibodies against both CVB4 and GAD65, and those with IgM Antibodies against both CVB3 and GAD65 were detected in T1D patients, 14 (23.3%) and 4 (6.7%), respectively; and in healthy controls 4 (3.3%) and 6 (5.0%), respectively. T1D was significantly associated with those group of patients who carry IgM Antibodies against both CVB4 and GAD65 (OR 8.8 [95%CI = 2.7 – 28.2], $p = 0.0002$) and was not significantly associated with those group of patients who carry IgM Antibodies against both CVB3 and GAD65 (OR 1.3 [95%CI = 0.3 – 5.0], $p = 0.6$).

Out of the T1D patients, 8 (13.3%) and 13 (21.6%) were positive for IgG Antibodies against both CVB4 and GAD65, and those with IgG Antibodies against both CVB3 and

GAD65, respectively. Of controls, 6 (5.0%) were positive for IgG Antibodies against both CVB4 and GAD65 and 1 (0.83%) were positive for those with IgG Antibodies against both CVB3 and GAD65, respectively. T1D was not significantly associated with those group of patients who carry IgG Antibodies against both CVB4 and GAD65 (OR 0.9 [95%CI = 2.9 – 8.8], $p = 0.05$) and was significantly associated with those group of patients who carry IgG Antibodies against both CVB3 and GAD65

(OR 32.9 [95%CI = 4.2 – 258.7], $p = 0.0009$).

GAD65 IgM and IgG Autoantibodies against beta cells of islet were detected in 21 (35.0%) and 23 (38.3%), respectively, of T1D patients and detected in 49 (40.8%) and 6 (5.0%), respectively, of healthy controls. The GAD65 IgM was not significantly associated with T1D (OR 0.7 [95%CI = 0.4 – 1.5], $p = 0.7$). GAD65 IgG was significantly associated with T1D (OR 11.8 [95% CI = 4.4 – 31.2], $p = 0.001$). These findings are presented in Table (1).

Table 1: Multiple analysis of IgG, IgM against CB3, CB4, GAD65 Risk factors of newly Diagnosed T1DM infection, unmatched case-control study.

Antibody & PCR results	T1DM Cases N= 60 (%)	Control N= 120 (%)	Matched Odds Ratio	95% CI	P- value
Male	34 (56.7%)	68 (56.7%)			
Female	26 (43.3%)	52 (43.3%)			
CVB4 IgM	17/60 (28.3%)	1/120(0.83%)	47	6.1- 364.3	0.0002
CVB3 IgM	8/60 (13.3%)	10/120 (8.3%)	1.7	0.6 – 4.5	0.2
CB4 IgG	16/60 (25%)	18/120 (15%)	2.1	0.9 – 4.4	0.06
CVB3 IgG	31/60 (51.67%)	54/120 (45%)	1.3	0.7 – 2.4	0.3
CVB4 RNA Positive	15/60(25.0%)	1/120 (0.83%)	39.6	5.1 –309	0.0004
CB3 RNA Positive	9/60 (15.0%)	21/120 (17%)	0.8	0.3 – 1.9	0.6
IgM Antibodies against both CVB4 and GAD65	14/60 (23.3%)	4/120 (3.3%)	8.8	2.7 –28.2	0.0002
IgM Antibodies against both CVB4 and GAD65	4/60 (6.7%)	6/120 (5.0%)	1.3	1.3-0.9	0.6
IgG Antibodies against both CVB4 and GAD65	8/60 (13.3%)	6/120 (5.0%)	2.9	0.9 – 8.8	0.05
IgG Antibodies against both CVB3 and GAD65	13/60 (21.7%)	1/120 (0.83%)	32.9	4.2- 258.7	0.0009
GAD65 IgM Positivity	21/60 (35.0%)	49/120(40.8%)	0.7	0.4 – 1.5	0.7
GAD65 IgG Positivity	23/60 (38.3%)	6/120 (5.0%)	11.8	4.4 – 31.2	0.0001

DISCUSSION

Evidence of a role for viral infection in the development of T1D appears from epidemiological studies that showed an increased incidence of T1D after enterovirus epidemics (Wagenknecht *et al.*, 1991). This study was carried out after the presumptive epidemic of enteroviruses during and after conflicts (violence, displacement, crowding and unhygienic situation) which enhanced transmission of enteroviruses.

This study revealed that CVB4-IgM and CVB4 RNA were significantly associated with T1D ($p = 0.0002$ and 0.0004 , respectively). This finding is consistent with

that in the literatures (Salminen *et al.*, 2003; Al-Suhail *et al.*, 2003; Graves *et al.*, 2003; Coppieters *et al.*, 2012; Hober *et al.*, 2013;) Detection of IgM and RNA define the infection.

T1D might be explained by the fact that the relation of enterovirus infection with T1D is not consistent in all studies (Stene *et al.*, 2010). It might be attributed to the geographical difference, also.

It was observed that 40% of T1D patients had CVB RNA. The Diabetes and Autoimmunity Study in Young (DAISY) reported that 8% of children progressing to T1D had enteroviral RNA (Stene *et al.*,

2010). The difference might be explained by the difference in the design of studies. The DAISY is a cohort prospective study and this study is a case-control study.

Enterovirus is normally present in blood for only a few days during infection of an immunocompetent host, and so the time of sampling affects the finding. Another possible explanation is in the fact that enterovirus may establish low-grade persistent infection in children with islet autoimmunity but the quantity of viral RNA in serum may be below the detection limit (Almahhfoodh *et al.*, 2017).

In the line with that reported in Baghdad (Al-Suhail 2003) autoimmunity (IgM antibodies against both CVB4 and GAD65 and also IgG antibodies against both CVB3 and GAD) was significantly associated with T1D. DAISY (Stene and Rewers 2012) reported similar findings.

The study showed that CVB3-IgM and IgG antibodies against both CVB4 and GAD65 were not associated with T1D. This finding might be explained by the difference in sampling time. Some of T1D cases were selected from the emergency unit as they presented for the first time in ketoacidosis, hyperglycemia or hypoglycemia i.e. the T1D might be initiated a time before selection. However, this study showed the autoimmunity to a beta cell of islet-associated with T1D like that reported in the literatures (Salminen *et al.*, 2003; Al-Suhail 2003; Hober *et al.*, 2013).

In our work coxsackievirus antibodies had been detected with ELISA, the present of CB4 IgM Positivity and CB4 RNA Positive were significantly associated with newly diagnosed T1DM illness and at higher risk of T1DM compared to non T1D Minfected healthy control group. These results were consistent with other studies that recently demonstrated coxsackie B4 virus demonstrated in pancreas β cells from patients with type1 diabetes (Van der Werf *et al.*, 2007; Dotta *et al.*, 2007; Oikarinen *et al.*, 2011; Stene and Rewers 2012; Hober *et al.*, 2013).

Because the frequency of CB4 infection was high in the controls with low incidence of juvenile diabetes, only a small proportion of children can be susceptible to diabetes, even in the face of a CB4 infection. Factors determining susceptibility to diabetes are unknown, but evidence that HL-A8 and W15 are unusually common in insulin-treated and juvenile diabetics suggests that immunological response or tissue susceptibility to specific viral infections might be related or linked to the same or adjacent genetic loci. Some authors have proposed that not all strains of a certain serotype of CBV are diabetogenic and they suggest that this is the reason why more children in a family do not develop diabetes (Dotta *et al.*, 2007; Hober and Sauter 2010).

Detection of CB3 and CB4 infection without development of T1DM during the current study was consistent with the previous study that showed that animal experiments have shown that a high variety in diabetogenicity of different CVB strains exists probably due to differences in tissue tropism of the virus (Ziegler *et al.*, 2013; Robertson 2015).

Several mechanisms for β cells dysfunction induced by Coxsackie B4 infection have been reported. Coxsackie B4 cytosolic infection may cause β cells lysis, which may expose self-antigens leading to the autoimmune response against β cells antigens (Knip and Siljander 2008; Marroqui *et al.*, 2015). Infection by Coxsackie B4 virus may also induce activation of T cells, which may directly cause β cells damage (Hodik *et al.*, 2016). Viral infection may also induce the release of inflammatory cytokines from β cells that can further stimulate the activation and infiltration of inflammatory cells (Berg *et al.*, 2006).

In addition, the autoimmune response to β cells induced by structural homology between viral protein epitopes and β cells antigens is also a well-known mechanism of autoimmune type 1 diabetes (Marttila *et al.*, 2002). Another mechanism may be that CBV causes alterations of the Beta cells,

which are recognized as foreign by the immune system. An autoimmune response could develop leading to the destruction of the β cells. This might also occur if antibodies induced by CBV react with human islet cell protein (Dotta *et al.*, 2007). The present study yields no information concerning the mechanisms involved.

In our work coxsackievirus antibodies had been detected with ELISA, This indicates the widespread of coxsackievirus B4 in Iraq and it may have a significant role in the causation of T1D. Similar results were found in the previous study carried also in Sudan's Showed 45% positive for IgG (Emad and Enan 2011) that indicate the high prevalence of coxsackievirus within T1D, another study conducted in Sweden in 1982; Found that 33% positive cases for IgM in T1D children (Åkerblom and Knip 1998).

Surprisingly, the current study revealed that infections by three other CB3 RNA Positive, GAD65 IgM Positivity and IgG Antibodies against both of CB4- GAD65 were associated with a decreased risk of β cells autoimmunity. A possible protective effect of CBV3 has actually been reported the previous study in a smaller study where patients with newly diagnosed type 1 diabetes were found to be less frequently positive for neutralizing antibodies against this serotype than control subjects (Tracy *et al.*, 2002; Drescher *et al.*, 2004; Bahri *et al.*, 2005; Serreze *et al.*, 2005; Schneider and von Herrath 2014). This phenomenon could be explained by immunological cross-protection induced by CB3 RNA Positive, GAD65 IgM Positivity and IgG Antibodies against CB4-GAD65 against the diabetogenic effect of CBV4 (Marttila *et al.*, 2002) .

Regarding the results of IgG Antibodies against both CB3 and GAD65 and IgM -Antibodies against both CB4-GAD65 that were significantly associated with illness and at higher risk of T1DM compared to non T1DM infected healthy control group, these results were in agreement with another report that showed mixed viruses with GAD65 can cause fulminant T1DM (Horwitz *et al.*, 2004) .

Mixed viruses with GAD65 that activate and impact each other can also aggravate the existing damage in a target tissue. Likewise, replicative cycles that result in "multiple hits" lead to recurrent and cumulative inflammation in target tissues (Schneider and von Herrath 2013).

On the other hand IgG Antibodies against both CB3 and GAD65 , and also IgM Antibodies against both CB4-GAD65 and GAD65 IgG Positivity on those group of patient who carries two types of antibodies were significantly associated with illness and at higher risk of T1DM compared to non T1DM infected healthy control group in current study, these results were in agreement with previous studies (Williams *et al.*, 2003; Schulte *et al.*, 2010) reported that combined analysis of GAD65 autoantibodies with CB3 and CB4 could increase the positive predictive value for type 1 diabetes in the general population.

Molecular mimicry effects between GAD and CB4, therefore, were suggested to play a role in islet cell destruction and the development of IDDM, After identification of the 64-kD islet antigen as GAD a sequence homology between this major autoantigen in diabetes and the 2C protein of CB4 was identified (Lönnrot *et al.*, 1996; Vreugdenhil *et al.*, 1998).

The present data indicate a significantly higher frequency of CB3 and CB4 RNA in type 1 diabetic children at the onset of disease in diabetic children as well as GAD65 autoantibody than in children of a control group. These results are in agreement with other studies including diabetic children, and support the hypothesis that different enteroviruses may be associated in the initiation of beta-cell destruction (Lonrot *et al.*, 2000; Salminen *et al.*, 2003).

Regarding molecular study using RT-PCR technique for detection of CB4 viral RNA in newly diagnosed T1DM, we found a significant statistical difference between patients and control. This agrees with (Andréoletti *et al.*, 1998; Nairn *et al.*, 1999) who found also significant statistical

difference between patients and control by RT-PCR in sera taken from newly diagnosed T1DM children and support evidence for the involvement of enteroviruses particularly Coxsackieviruses at the onset of newly diagnosed T1DM either as a primary etiologic agent or as a triggering factor (Hiltunen *et al.*, 1997).

On the other hand, in the current study, indirect ELISA for the detection of IgM for CVB4 detected only 17/60 (38.33%) positive cases corresponding to PCR that detected 15/60 (25%) positive cases. This discrepancy in results may be due to there was no stage of viremia in some patients. By indirect ELISA, there was significant statistical difference between cases and control, 17/60 38.33% (17 out of 60 cases) were positive in cases and 0.83% (one case out of 120) was positive in controls, and this may be due to the duration of the IgM response was reported to be between 6 and 8 weeks from onset of illness (Andréoletti *et al.*, 1998). Our results similar from findings in two Finnish prospective studies with a similar number of cases (Andréoletti *et al.*, 1998; Viskari *et al.*, 2012) which reported that PCR-defined EV including CB4 virus infection was present at a significantly higher frequency in cases than controls.

Regarding the results of RT-PCR, in addition to anti-CVB IgM and IgG antibodies were searched during current study by enzyme immunoassay were consistent with an Egyptian study (Ismail *et al.*, 2008) , A Japanese case-control study (Kawashima *et al.*, 2004) , A German group searched (Moya-Suri *et al.*, 2005) , Cuba study (Salminen *et al.*, 2003 and Sarmiento *et al.*, 2007) who showed, coxsackievirus B IgM antibodies and CBV RNA were significantly higher in newly diagnosed diabetic patients than those in healthy control group but the present study was disagreement with another study by a Swedish group (Yin *et al.*, 2002) that showed that showed the difference was not statistically significant. Between newly diagnosed T1DM with control group regarding frequency of enteroviruses

particularly coxsackievirus B using the same test.

CONCLUSION:

CVB3 and CVB4 are evolved as factors in the T1D.

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