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Serological characterization of Coxsackievirus B in type 1 diabetes mellitus (T1DM) patients in Basrah city- Iraq

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Abstract

Background: Type 1 diabetes mellitus (T1DM) is a chronic autoimmune illness that causes severe insulin shortage and hyperglycemia. Pancreatic cells may be destroyed by enteroviral (EV) infection either directly or by inducing an inflammatory response in the pancreatic islets that brings autoreactive T lymphocytes to the site of inflammation.

Aim of study: investigate whether coxsackievirus group B (CVB) strains are associated with the onset of islet autoimmunity and T1DM.

Objective: Serum samples were collected from newly diagnosed patients of type 1 diabetes mellitus who were attending the endocrinology centers at Al-Mawani general hospital and Al-Fayhaa teaching hospital from October 2022 to May 2023. A total of 168 children of both sexes, comprising 86 (51.1%) patients as a patient group.

Results and discussion: Showed that there were no significant differences ($p \leq 0.871$) between patients and control according to age range however, the results showed elevated IgM titers in patients (15.14 ± 2.43) in comparison with the control (8.66 ± 1.56) with highly significant differences ($p < 0.000$). The age group (11-17) had the highest percentage (57.58%) of positive CVB-IgM patients which was 19 children of the total 33 infected children, while other age groups had 7 patients each (21.21%).

Conclusion: Females were significantly more susceptible to T1DM than males. The age group (11-17) significantly more susceptible to T1DM than other age groups. Serological methods were more favorable than molecular methods in diagnosing the Coxsackievirus B. Elevated titers of IgG or IgM in patients in comparison with the control.

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Keywords: type 1 diabetes mellitus, CVB, IgG, Iraq, Basrah

Introduction

The pathophysiology and therapy of type 1 diabetes may appear to be easily manageable at first, but as more it is learned about the condition, it appears that less is actually understood. There isn't a single Koch's postulate that applies to all instances as a result of increased knowledge of the disease's pathophysiology. It is now known that what was once thought to be a single autoimmune disorder caused by a T-cell-mediated attack on insulin-producing cells was actually the result of a complex interactions between environmental factors as well as differences in each person's microbiome, genome, metabolism, and immune system ^[1]. The chronic condition known as type 1 diabetes mellitus (T1DM) is characterized by the body's inability to manufacture insulin as a result of the autoimmune destruction of the beta cells in the pancreas. Insulin is a crucial anabolic hormone that, in addition to affecting development, has multiple impacts on the metabolisms of glucose, lipids, proteins, and minerals. Thus, T1DM manifests as a systemic illness characterized by the hyperglycemia phenotype ^[2]. Viruses are thought to be important environmental variables that cause genetically susceptible people to develop autoimmune diseases ^[3]. There is epidemiological and experimental support for enteroviruses, particularly CVB, playing a role in the etiology of T1DM ^[4].

In recent years, there have been studies on the most important viruses that cause diseases in Basrah Governorate, as in studies Shihab *et al.*, 2020^[5], Mohaned *et al.*, 2020^[6], Al-Salait *et al.*, 2021^[7], Nasser and Al-Hmudi, 2021^[8] and Almarjan *et al.*, 2021^[9]. The purpose of this research is to make serological characterization of Coxsackievirus B in type 1 diabetes mellitus (T1DM) patients in different age groups.

Materials and Methods

Study subjects

This study was carried out from October 2022 to May 2023. Serum samples were collected from newly diagnosed patients of type 1 diabetes mellitus (before insulin therapy) who were attending the endocrinology centers at Al-Mawani general hospital and Al-Fayhaa teaching hospital. A total of 168 children from both sexes, comprising 86 individuals as patients' group, 33 of them are males and 53 females with age range 1-17 years. Furthermore, 82 children as a control group, 31 of them are males and 51 females with age range 1-17 years.

Ethical considerations were taken into account, and informed consent was obtained from all participants prior to sample collection.

A 5 ml of blood were collected by vein puncture from patient children at the onset of type 1 diabetes mellitus, some patients

experienced with other symptoms like fever and rash before the onset of the disease, and the same amount was collected from healthy children of approximate age and sex.

Determination of Coxsackievirus B IgM and B -IgG antibodies

All serum samples were examined to detect COX V type B -IgM and COX V type B -IgG. Determination was performed by using ELISA according to the manufacture manual of (SunLong Biotech Co., LTD, China).

Results

A total of 168 children of both sexes, comprising 86 patients as a patient group, 33 (38.3%), Furthermore, 82 healthy individuals as a control group, Current study showed that there were no significant differences ($p \leq 0.871$) between patients and control according to age range (Table,1; Figure,1). Statistically, the age group (11-17) had the highest percentage (59.30%) of children with T1DM than other age groups with highly significant differences ($p < 0.000$). The age group (11-17) had the highest percentage (12/23; 52.17% and 14/21; 66.66%) of fever and rash, respectively with highly significant differences ($p < 0.000$) than other age groups whose percentages were (5/23; 21.75% for fever; 3/21; 14.38% for rash, and 6/23; 26.18% for fever; 4/21; 19.06% for rash) of (1-5) and (6-10) age groups, respectively.

Table 1: Distribution of patients and control groups according age

Groups	Age (years) \ (No., %)			p-Value
	G1(1-5)	G2(6-10)	G3(11-17)	
Patients	20 / (23.26%)	15 / (17.44%)	51 / (59.30%)	Patients vs Control (Age) ≤ 0.871
Control	17 / (20.73%)	15 / (18.29%)	50 / (60.98%)	

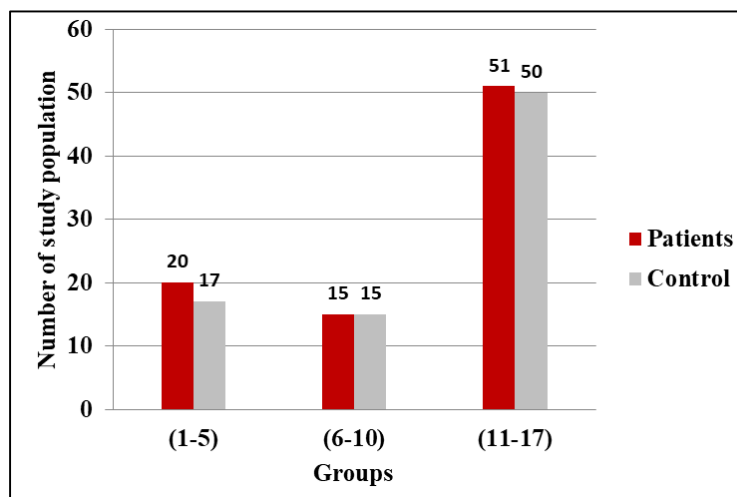


Fig 1: Distribution of patients and control according to age range

IgM +ve and serum titers among age groups

In comparison to healthy individuals in the same age groups, statistical analysis revealed that patients of the (1-5), (6-10) and (11-17) age groups had significantly higher Coxsackievirus B IgM antibodies ($p \leq 0.000$).

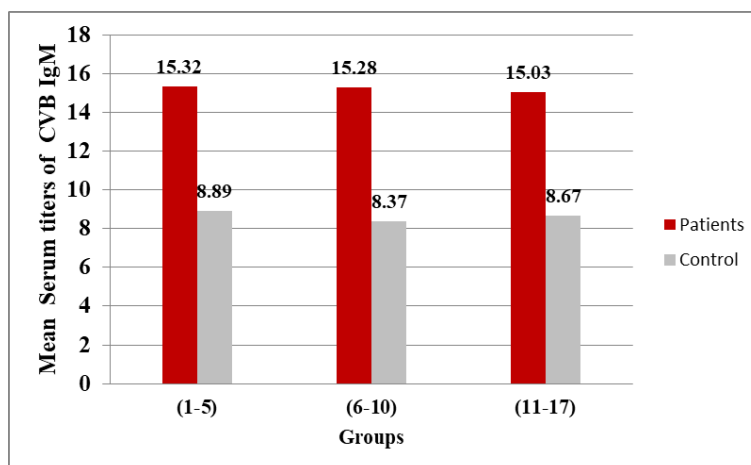
Statistically, the age group (11-17) had the highest percentage (57.58%) of positive CVB-IgM patients which was 19 children of the total 33 infected children, while other age groups had 7 patients each (21.21%). In fact, according to percentage of IgM-positive within each group, the age group (6-10) years had the highest percentage 7/15 (46.6%) of IgM-positive with highly significant differences ($p \leq 0.000$) than

other groups. In addition, the lowest percentage was within the age group (1-5) years, which was 7/20 (35%).

Also, the age groups did not show any significant differences between them regarding the IgM-positive levels. (Table 2; Figure 2). The age group (11-17) had the highest percentage (9/17; 52.94% and 11/16; 68.75%) of fever and rash, respectively with highly significant differences ($p < 0.000$) than other age groups whose percentages were (4/17; 23.53% for fever; 2/16; 12.5% for rash, and 4/17; 23.53% for fever; 3/16; 18.75% for rash) of (1-5) and (6-10) age groups, respectively.

Table 2: Serum titers of human Cocksackie virus B IgM in patients and control of studied age groups

Groups	P value			Age (years) (IgM +ve\ Mean± SD)			P value		
				G1(1-5)	G2(6-10)	G3(11-17)			
Patients	G1\ Patients vs. Control	G2 \ Patients vs. Control	G3 \ Patients vs. Control	7/15. 32±2.27	7/ 15. 28±2.77	19/ 15. 03±2.43	G1 Patients vs. Control	G2 Patients vs. Control	G3 Patients vs. Control
Control	(Mean± SD)= 0.000	(Mean± SD)= 0.000	(Mean± SD)= 0.000	0/8.89 ±1.58	0/8.37 ±1.33	0/8.67 ±1.63	(IgM +ve)= 0.000	(IgM +ve)= 0.000	(IgM +ve)= 0.000

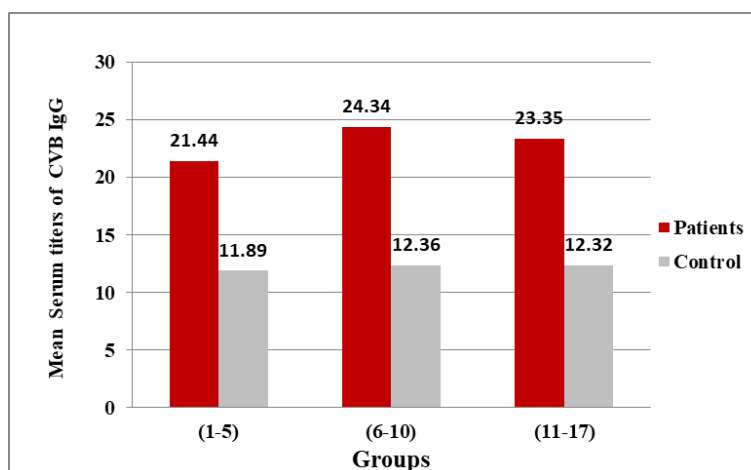
**Fig 2:** Mean serum titers of human Cocksackie virus B IgM in studied age groups

2. IgG +ve and serum titers among age groups

Statistical analysis showed that the positive Cocksackievirus B IgG antibodies in patients with (1-5), (6-10) and (11-17) age groups which elevated significantly ($p \leq 0.000$) than control group of the same age groups (Table 3; Figure 3). Statistically, the age group (11-17) had the highest percentage (52.18%) of positive CVB- IgG patients which was 12 children of the total 23 infected children with highly significant differences ($p < 0.000$) than other groups whose percentages were 62.08% and 21.74% for (1-5) and (6-10) groups, respectively.

Also, according to the percentage of IgG-positive within each

group, the lowest percentage was within the age group (11-17) years, which was 12/51 (23.52%) with a significant decreased ($p \leq 0.000$) than other groups whose percentages were 30 % and 3.33% for (1-5) and (6-10) age groups, respectively. Also, the age groups did not show any significant differences between them regarding the IgG - positive levels. The age group (11-17) had the highest percentage (7/10; 70% and 5/7; 71.42%) of fever and rash, respectively with highly significant differences ($p < 0.000$) than other age groups whose percentages were (1/10; 10% for fever; 1/7; 14.29% for rash, and 2/10; 20% for fever; 1/7; 14.29% for rash) of (1-5) and (6-10) age groups, respectively.

**Fig 3:** Mean serum titers of human Cocksackie virus B IgG in studied age groups

Discussion

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune condition caused by an insulin deficiency that results from the loss of pancreatic islet cells [10]. A role for viral infections, particularly those caused by enteroviruses like CVB, is supported by environmental factors and epidemiological, clinical, and pathological research [11]. The recent study demonstrated that the age had no effect on the both studied

groups (patients and control), In AL-Zobaei *et al.*, 2019 [12], study documented that. No significant difference in age was noticed between cases of T1DM and healthy control.

About other symptoms like fever and rash, 23/86 (26.74%) and 21/86 (24.41%) patients experienced with fever and rash, respectively. The above findings agreed with Sekine *et al.*, 2001 [13] study who reported that the pancreatic β -cells are destroyed within just a few days in case of fulminant type 1

diabetes. Studies presenting reports on nationwide surveys have revealed that flu-like symptoms were observed in about 71.2% of patients with fulminant type 1 diabetes (FT1DM). This suggests that viral infection was definitely critical in the development of fulminant type 1 diabetes (FT1DM). Among the other symptoms, fever was the most frequent and was observed in 60.0% of patients^[14]. Also, Caffarelli *et al.*, 2004^[15] study who recorded an itchy rash in 9 (14.3%) of children with T1DM.

The current study has confirmed positive anti-CVB- IgM results of serum within 86 patients, 33/86 (38.37%), which highlighted the presence of a recent infection of CVB accompanied with T1DM fulminant. However, these findings agreed with Banatvala *et al.*, 1985^[16] with a slightly higher percentage, 37 /122 (30%) of children within age below 15 years old. In the Hiltunen *et al.*, 1997^[17] study who reported that the frequency of CVB IgM antibodies has been found to be higher in diabetic children. In this study, the age group (11-17) had the highest percentage (57.58%) of positive CVB-IgM patients with highly significant differences ($p \leq 0.000$) than other groups. However, these findings agreed with AL-Zobaei *et al.*, 2019^[12], study who proposed, that children aged less than 17 years are at risk of T1DM infection if exposure to CVB4.

The present results showed elevated CVB-IgG titers in patients in comparison with the control with highly significant differences.

In this study, the age group (11-17) had the highest percentage (52.18%) of positive CVB- IgG patients with highly significant differences than other groups, which was disagreed with Kareem *et al.*, 2020^[18] study who documented that the highest mean of anti-CVB -IgG positive was in children with age group (1–5) years, which was disagreed with Kareem *et al.*, 2020^[18] study who documented that the highest mean of anti-CVB -IgG positive was in children with age group (1–5) years.

Conclusion

Females were significantly more susceptible to T1DM than males. The age group (11-17) significantly more susceptible to T1DM than other age groups. Serological methods were more favorable than molecular methods in diagnosing the Cocksackievirus B. Elevated titers of IgG or IgM in patients in comparison with the control. The results of GAD or IA-2 levels in patients were higher than those of control.

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