Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed.nahrainuniv.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2022; Vol. 20(1)

Association of Anti-Coxsackie Virus-B IgG with Autoantibodies Related to Type 1 Diabetes Mellitus

Hakeem Gh. Hassan[1] *MSc*, **Arwa M. Al-Shuwaikh**[2] *PhD*, **Raid K.D. Al-Auqili**[3] *FIBMS (Ped)*

[1]Al-Hussein Teaching Hospital, Thi-Qar, Iraq, [2]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [3]Dept. of Pediatrics, College of Medicine, Thi-Qar University, Thi-Qar, Iraq

Abstract

Background Coxsackievirus B is a virus may cause type 1 diabetes. There are links between Coxsackievirus B infections and type 1 diabetes. The presence of autoantibodies in pancreatic beta cells has been linked to the development of type 1 diabetes following Coxsackievirus B infection.

Objective To detect autoantibodies in patients with type 1 diabetes. Also, to find if there is an association between pancreatic beta cell autoantibodies and Coxsackievirus-B IgG.

Methods This study was done from January to March 2021, it included two groups of 75 children; their ages ranged from one month to fifteen years. Children with type 1 diabetes were admitted to the diabetic and endocrine glands center in Thi-Qar governorate, whereas children without diabetes (control group) were admitted to Bint Al-Huda Children's Hospital in Nasiriyah/Thi-Qar. Venous blood was taken from each person for estimation of random blood sugar, serum fructosamine, and HbA1c in the laboratory of the diabetic and endocrine glands center. Pancreatic beta cell autoantibodies and anti-Coxsackievirus-B IgG was detected by enzyme-linked immunosorbent assay. The statistical analyses were done using SPSS 25. The study population P-values below 0.05 to be statistically significant. The study was authorized by Al-Nahrain University's Institutional Review Board and parental consent was taken.

Results The patients had significantly greater levels of random blood sugar, fructosamine, and HbA1c than healthy controls. Anti-islet antigen, anti-islet cell, anti-glutamic acid decarboxylase, and anti-Coxsackievirus-B IgG antibody titers were greater in patients than controls. The majority of autoantibodies tested correlated with Coxsackievirus-B IgG antibodies.

Conclusion Anti-Coxsackievirus-B IgG antibody positivity was associated with autoantibodies related to type 1 diabetes mellitus.

Keywords Coxsackievirus B, type 1 diabetes, autoantibodies

Citation Hassan HG, Al-Shuwaikh AM, Al-Auqili RKD. Association of Anti-Coxsackie Virus-B IgG with autoantibodies related to type 1 diabetes mellitus. Iraqi JMS. 2022; 20(1): 132-138. doi: 10.22578/IJMS.20.1.17

List of abbreviations: CVB = Coxsackievirus B, DCM = Dilated cardiomyopathy, ELISA = Enzyme-linked immunosorbent test, FBS = Fasting blood sugar, GADA = Glutamic acid decarboxylase 65 autoantibodies, HbA1c=hemoglobin A1c, HEVs = Human enteroviruses, HFMD = Hand-foot-and-mouth disease, IA-2A = Tyrosine phosphatase–like insulinoma antigen-2 antibodies, IAA = Insulin autoantibodies, T1DM = Type 1 diabetes mellitus, ZnT8 = Zinc transporter 8 autoantibodies

Introduction

Coxsackievirus B (CVB) is classified as an enterovirus of the Picornaviridae family. CVB is a single-stranded RNA virus that infects humans [(1)]. CVB viruses are classified into six serotypes, designated as CVB1–6, and in the majority of cases, infections with these viruses are asymptomatic or result in mild symptoms that are similar to those of the

common cold or flu [2]. CVB, on the other hand is linked to several of potentially life-threatening disorders, including encephalitis and aseptic meningitis [1], myocarditis and chronic dilated cardiomyopathy (DCM) [3], pancreatitis [2], and hand, foot, and mouth disease (HFMD) [4]. There are also substantial connections between CVB infections and the chronic autoimmune illness type one diabetes (T1DM), showing that these viruses are a significant contributor to an expanding range of diseases with significant clinical and economic consequences [5].

Specific CVB serotypes are shown to be related to each of the disorders listed above. As an example, CVB3 infections are linked to viral myocarditis and DCM [6], CVB5 infections are linked to encephalitis and aseptic meningitis outbreaks [7], and CVB1 is linked to the induction of β-cell autoimmunity, which is linked to T1DM [8]. In type one diabetes, beta cells are destroyed by an antibody that affects the pancreas, leading to the loss of insulin-producing beta cells [9].

Diagnosing T1DM from other forms is particularly important in its treatment because it helps with medication selection, evaluation of illness prognosis, and determining the risk of diabetes development in family members of the diabetic patient. T1DM is distinguished from other forms by the presence or absence of insulin production [8].

A large number of autoantibodies have been discovered in pancreatic beta cells, and it has been postulated that these autoantibodies may act significant roles at the beginning of autoimmune islet destruction [7]. The autoantibodies that have developed as a result of islet destruction are most likely directed towards insulin itself as their principal target. Glutamic acid decarboxylase 65 autoantibodies (GADA), insulin autoantibodies (IAA), and zinc transporter 8 autoantibodies (ZnT8) may be utilized to diagnose T1DM [7].

People who have just been diagnosed with T1DM are nearly always found to have one or more autoantibodies at the time of their diagnosis, which is virtually always accurate [4]. The prevalence of type one diabetes in newly diagnosed individuals is increasing. The presence of islet cell autoantibodies (ICA) is discovered in 85 percent of cases [5], the presence of GADA is detected in 70%, and the presence of Tyrosine phosphatase–like insulinoma antigen-2 antibodies (IA-2A) is detected in 58% of patients [6].

The objectives of this study were to identify people who have autoantibodies associated with T1DM and to determine whether or not there is a correlation between autoantibodies against pancreatic beta cells and IgG against coxsackievirus-B.

Methods

Blood sample was taken from a total of 75 patients (38 males + 37 females), that were recently diagnosed with T1DM at the Center of Diabetic and Endocrine Glands in Thi-Qar Governorate throughout three-month period from January to March 2021. In addition, 75 control blood samples (31 males + 44 females), were collected from children visiting Bint Al-Huda Children's Hospital. Blood samples were collected from non-diabetic children who seemed healthy and had no family history of T1DM (left over). This group of people included boys and females who were in the same age range as the patient population (ranging from one month to 15 years).

Using a disposable syringe, venous blood was taken, providing a volume of 5 ml. Each blood sample was separated into two parts using a sterile plane tube. In part one, 3 ml of blood were drawn and left to coagulate at room temperature in order to separate the serum from the remainder of the sample.

The enzyme-linked immunosorbent assay (ELISA) was used to evaluate anti-CoxV-B IgG levels and autoimmune antibodies (by using kits manufactured in Shenzhen New Industries/China) in serum that had been stored at -20°C. The quantitative measurement of anti-CoxV-B IgG was developed using the cut off value as a reference. In order to determine HbA1c levels, the remaining 2 ml of blood were

drawn and immediately deposited into an Ethylene Diamine Tetra Acetic Acid (EDTA) tube for further processing.

Statistics were carried out with the aid of the statistical package for the social sciences (SPSS) software version 25. The mean and standard deviation of data with a normal distribution, as well as the T test, were calculated. If data having a non-normal distribution were significant, the Mann Whitney U test was used to examine the median and range of each variable. When used in conjunction with a receiver operating characteristic curve (ROC), anti-CoxV-B IgG has been shown to be diagnostically beneficial in the discrimination between patients and controls. It was determined that a statistically significant difference existed when the P-value was less than 0.05, according to the findings of the research.

Results

Biochemical tests related to T1DM

Fasting blood sugar (FBS) and HbA1c were significantly greater in patients (268.92±86.21 mg/dl and 8.23±1.76%, respectively) than in controls (113.94±17.08 mg/dl and 4.76±0.5%, respectively) (P<0.001). Also, patients had greater levels of fructosamine than controls (5.42±1.0 mmol/l versus 1.91±0.18 mmol/l), (Table 1).

Table 1. Biochemical tests related to T1DM

Tests		Patients (n=75)	Control (n=75)	p value☐
FBS (mg/dl)	Mean±SD Range	268.92±86.21 135-560	113.94±17.08 84-158	< 0.001
Fructosamine (mmol/l)	Mean±SD Range	5.42±1.0 3.8-7.9	1.91±0.18 1.7-2.5	< 0.001
HbA1c, %	Mean±SD Range	8.23±1.76 6.7-12.7	4.76±0.5 4.2-6.1	< 0.001

☐Student t-test

Autoantibodies tests related to T1DM

According to table (2), the median levels of anti-Islet antigen antibody (Anti-IA2), islet cell antibody (ICA), and glutamic acid decarboxylase antibodies (GAD65) in patients were 34.8 U/ml, 32.7 U/ml, and 43.8 IU/ml, respectively, compared to 16.9 U/ml, 17.3 U/ml, and 14.8 IU/ml in controls with highly significant differences.

Detection of anti-CoxV-B IgG antibody

Anti-CoxV-B IgG antibodies titer in patients was 0.85 pg/ml (range 0.25-3.3 pg/ml), which was higher than the median serum level in controls (median= 0.71 pg/ml, range0.47-0.97 pg/ml), showing that patients had higher levels of antibodies than controls (p=0.005). Ten patients (13.33%) tested positive for anti-CoxV-B IgG antibodies, while none of the children in the control group tested positive, according to the manufacturer's recommendations of kit (Figure 1).

Table 2. T1DM-Related autoantibodies

Autoantibodies		Patients (n=75)	Control (n=75)	P value☐
Anti-IA2 (U/ml)	Mean±SD	38.2±10.16	16.16±5.45	< 0.001
	Median	34.8	16.9	
	Range	25.9-71.2	6.8-27.3	
ICA (U/ml)	Mean±SD	34.31±6.35	16.27±5.19	< 0.001
	Median	32.7	17.3	
	Range	24.9-59.6	7.9-27.0	
GAD (IU/ml)	Mean±SD	49.56±13.07	15.36±4.84	< 0.001
	Median	43.8	14.8	
	Range	31.6-85.2	7.9-28	

☐Mann Whitney U test

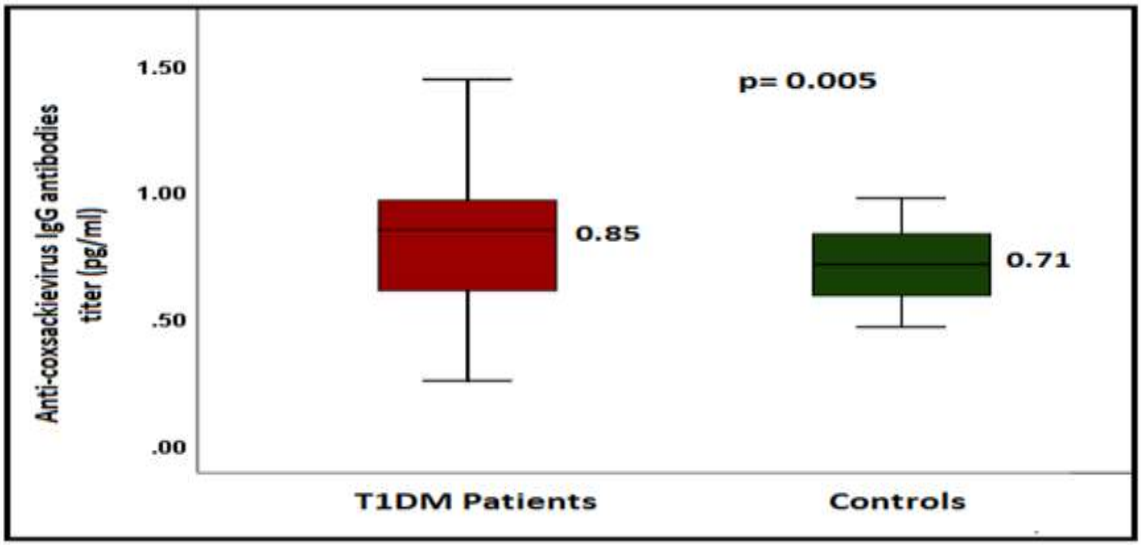

Figure 1. Median level of anti-Coxsackievirus-B IgG antibodies titer in T1DM patients and controls

Association of anti-CoxV-B IgG antibody positivity with autoantibodies tests related to T1DM

Most autoantibodies tests showed significant association with anti-CoxV-B IgG antibody. The median level of anti-IA2, ICA and GAD65 in patients positive for anti-CoxV-B IgG antibodies was 53.15 U/ml, 41.75 U/ml and 58.6 U/ml, respectively compared with 34.6 U/ml, 31.9 U/ml and 43.7 U/ml, respectively, in patients negative for anti-CoxV-B IgG antibodies with highly significant differences (Table 3).

Table 3. Association of anti-Coxsackievirus-B IgG antibodies positivity with autoantibodies tests related to T1DM

Autoantibodies		IgG-positive (n=10)	IgG-negative (n=65)	p- value
Anti-IA2 (U/ml)	Median Range	53.15 87.8-71.2	34.6 25.9-59.8	< 0.001☐
ICA (U/ml)	Median Range	41.75 36.5-59.6	31.9 24.9-41.9	< 0.001☐
GAD (IU/ml)	Median Range	58.6 41.6-74.9	43.7 31.6-85.2	0.013☐

☐Mann Whitney U test

Discussion

In this study, researchers found a statistically significant positive connection between FBS, HbA1c, and fructosamine in persons with T1DM. Like our findings, Zamanfar et al. in Iran (9), 10. Basu et al. in India [10], Belhiba et al. in Morocco [11], have reported positive correlations between FBS, HbA1c, and fructosamine values with diabetic patients.

According to the data, HbAlc has been found to be a strong predictor of fructosamine. On the other hand, FBS and fructosamine have been found to be strong predictors of HbA1c. Fructosamine offers a blood sugar state in 2-3 weeks, but HbA1c provides a blood sugar status in 2-3 months and is inconsistent with red blood cell lifespan (RBCs). Younger people with fewer red blood cells had lower HbA1c levels, whereas older people with more RBC have higher HbA1c values [12].

T1DM had greater levels of anti-IA2, anti-ICA, and GAD65 antibodies than healthy controls. Zamanfar et al. in Iran [9], Basu et al. in India [10], Belhibaet al. in Morocco [11], and Bravis et al. in the United Kingdom [13] all came to a similar result.

Beta cell autoimmunity is defined by the presence of diabetes-associated autoantibodies in the bloodstream, which indicates that the cells are being attacked. It is necessary to differentiate between diabetes types 1, 2, and monogenic diabetes using these indicators, which are crucial diagnostic tools. Among the signs and symptoms of T1DM include the existence of GAD65, anti-IA-2, ICA, and ZnT8 autoantibodies [9].

Despite the fact that most persons with a single autoantibody do not develop T1DM, children with two or more serum autoantibodies have an 84% chance of having the condition by the age of eighteen. Because many autoantibodies enhance the risk of progression, the stages of T1DM have been reclassified and reinterpreted [14].

The present study found that T1DM patients had higher levels of anti-CoxV-B IgG than the control group. These findings matched those of Kareem et al. [15], but not those of Bilal et al. [16] who found no differences between anti-CoxV-B IgG in T1DM patients and the control group. A negative anti-CoxV-B IgG test does not rule out a current or recent viral infection. Insufficient IgG antibody levels in the samples may have been acquired too early in the illness development.

Enteroviruses, such as Coxsackievirus, may begin or accelerate the process, leading to clinical T1DM. Viral-induced cytolysis of pancreatic beta-cells may cause direct cell death [16]. Instead, a less aggressive enterovirus infection may cause an inflammatory reaction in the islets, harming beta-cells or triggering an autoimmune response. Enterovirus-induced - cell damage may be produced by molecular mimicry of the islet cell protein due to homologous regions in both enteroviral and islet cell proteins [17].

Positive anti-CoxV-B IgG antibody findings were related to anti-IA2, ICA, and GAD65 in T1DM

patients, compared to negative anti-CoxV-B IgG antibody results. Results of Bilal et al. [16] and Sayah et al. [17], were similar to present the study. T1DM incidence increases following enterovirus outbreaks, suggesting a viral involvement in disease development. Human enteroviruses (HEVs), particularly the Coxsackievirus-B family, have been linked to Beta cell death [18].

Several mechanisms underpinning Coxsackie B4-induced cell dysfunction have been found and explored. Cytosolic infection with Coxsackie B4 may cause cell lysis, revealing self-antigens, and triggering an autoimmune response against cellular antigens. The Coxsackie B4 virus may also activate T lymphocytes, causing direct damage to β-cells. A viral infection may also cause T cells to produce pro-inflammatory cytokines, increasing the body's inflammatory cell activation and infiltration [19].

An extra advantage is an autoimmune response against beta cells caused by structural similarities between viral protein epitopes and cellular antigens. Another theory is that CVB causes alterations in Beta cells, which the immune system mistakenly assaults. An autoimmune response might cause cell death. This might happen if CVB autoantibodies react with a protein present in human islet cells [16].

In conclusions, anti-CoxV-B IgG IgG antibody positivity was associated with autoantibodies related to T1DM. Also, in CVB-T1DM patients, the presence of (anti-IA2, ICA, and GAD65) suggests that CVB viruses may have a role in the development of autoimmune disorders such as T1DM.

Acknowledgement

Authors would like to thank the laboratory staff in the Blood Collection Unit in Diabetic and Endocrine Glands Center and Bint Al-Huda Children's Hospital in Thi-Qar governorate for their cooperation in accomplishing this study.

Author contribution

All authors contributed to the study's planning, design, analysis, and interpretation.

Conflict of interest

Authors declare that there is no conflict of interest.

Funding

Self-funding.

References

1. Abedi GR, Watson JT, Nix WA, et al. Enterovirus and Parechovirus Surveillance - United States, 2014-2016. MMWR Morb Mortal Wkly Rep. 2018; 67(18): 515-8. doi: 10.15585/mmwr.mm6718a2.
2. Hong J, Kang B, Yeo S, et al. Pathogenesis of coxsackievirus B2 in mice: characterization of clinical isolates of the coxsackievirus B2 from patients with myocarditis and aseptic meningitis in Korea. J Vet Sci. 2017; 18(4): 457-64. doi: 10.4142/jvs.2017.18.4.457.
3. Hyöty H, Leon F, Knip M. Developing a vaccine for type 1 diabetes by targeting coxsackievirus B. Expert Rev Vaccines. 2018; 17(12): 1071-83. doi: 10.1080/14760584.2018.1548281.
4. Richardson SJ, Morgan NG. Enteroviral infections in the pathogenesis of type 1 diabetes: new insights for therapeutic intervention. Curr Opin Pharmacol. 2018; 43: 11-19. doi: 10.1016/j.coph.2018.07.006.
5. Dunne JL, Richardson SJ, Atkinson MA, et al. Rationale for enteroviral vaccination and antiviral therapies in human type 1 diabetes. Diabetologia. 2019; 62(5): 744-53. doi: 10.1007/s00125-019-4811-7.
6. Stone VM, Hankaniemi MM, Laitinen OH, et al. A hexavalent Coxsackievirus B vaccine is highly immunogenic and has a strong protective capacity in mice and nonhuman primates. Sci Adv. 2020; 6(19): eaaz2433. doi: 10.1126/sciadv.aaz2433.
7. Simmons KM, Youngkin E, Alkanani A, et al. Screening children for type 1 diabetes-associated antibodies at community health fairs. Pediatr Diabetes. 2019; 20(7): 909-14. doi: 10.1111/pedi.12902.
8. Zamanfar D, Yazdani P, Aarabi M, et al. The prevalence of type 1 diabetes in children of Mazandaran province. Iran J Health Sci. 2018; 6(2): 1-10.
9. Zamanfar D, Aarabi M, Amini M, et al. Prevalence of autoantibodies in type 1 diabetes mellitus pediatrics in Mazandaran, North of Iran. J Ped Endocrinol Metab. 2020; 33(10): 1299-305. doi: 10.1515/jpem-2019-0396.
10. Basu M, Pandit K, Banerjee M, et al. Profile of auto-antibodies (disease related and other) in children with type 1 diabetes. Indian J Endocrinol Metab. 2020; 24(3): 256-9. doi: 10.4103/ijem.IJEM_63_20.
11. Belhiba O, Aadam Z, Jeddane L, et al. Research of anti-GAD and anti-IA2 autoantibodies by ELISA test in a series of Moroccan pediatric patients with diabetes type 1. Afr Health Sci. 2020; 20(3): 1337-43. doi: 10.4314/ahs.v20i3.40.

12. Bala M, Meenakshi, Aggarwa S. Assessment of fructosamine levels prediabetes. Int J Healthcare Biomed Res. 2020; 8(3): 5-10. doi: 10.36848/IJHBR/2020/12100.51200.
13. Bravis V, Kaur A, Walkey HC, et al. Relationship between islet autoantibody status and the clinical characteristics of children and adults with incident type 1 diabetes in a UK cohort. BMJ open. 2018; 8(4): e020904. doi: 10.1136/bmjopen-2017-020904.
14. DiMeglio LA, Evans-Molina C, Oram RA. Type 1 diabetes. Lancet. 2018; 391(10138): 2449-62. doi: 10.1016/S0140-6736(18)31320-5.
15. Kareem AS, Almola GA, Alsalihi OJ. Molecular detection to type 1 diabetes-coxsackievirus B (T1D-CVB) patients in Hilla province. Sys Rev Pharm. 2020; 11(12): 1620-6. doi:10.25258/ijpqa.11.2.2.
16. Bilal RM, AL-Zobaei MA, AL-Ani ZR. Relation of Coxsackie B3 and B4 viral infections for development of type 1 diabetes mellitus in children: A case-control study. Egypt Acad J Biol Sci, G. Microbiol. 2019; 11(1): 1-12. doi: 10.21608/eajbsg.2019.25575.
17. Sayah MA, Mezher MN. Correlation between the levels of Coxsackie Virus B IgG antibody with the glutamic acid decarboxylase auto antibodies and with pro-inflammatory cytokines in type 1 diabetes mellitus patients. Indian J Public Health Res Develop. 2019; 10(8): 1169-73.
18. Richardson SJ, Morgan NG. Enteroviral infections in the pathogenesis of type 1 diabetes: new insights for therapeutic intervention. Curr Opin Pharmacol. 2018; 43: 11-19. doi: 10.1016/j.coph.2018.07.006.
19. Isaacs SR, Foskett DB, Maxwell AJ, et al. Viruses and type 1 diabetes: From enteroviruses to the virome. Microorganisms. 2021; 9(7): 1519. doi: 10.3390/microorganisms9071519.

Correspondence to Hakeem Gh. Hassan
E-mail: alhamamy201567@gmail.com
Received Dec. 28th 2021
Accepted Apr. 6th 2022