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Serological and Metabolic Test Correlates of Coxsackievirus B Infection in Iraqi Children with Type 1 Diabetes

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Abstract

The autoimmune condition identified as type 1 diabetes mellitus (T1DM) has been associated to the death of pancreatic β-cells. In this study, Iraqi children with type 1 diabetes had their metabolic and serological signs of CVB infection evaluated. Techniques: From the end of 2023 to the beginning of 2024, Al-Diwaniyah Teaching Hospital carried out a case-control study. 56 youngsters with T1DM who received clinical diagnoses between the ages of 3 and 15 were included. qRT-PCR targeting VP1 was used to detect CVB RNA. Enzymatic and HPLC techniques were used for calculating glycated hemoglobin (HbA1c), random blood sugar (RBS), and fasting serum glucose (FSG). ELISA was used to identify anti-glutamic acid decarboxylase (anti-GAD) antibodies.

Results: 20/56 (35.7%) samples had CVB RNA. RBS (268.9 ± 37.19 vs. 223.47 ± 30.23 mg/dL, p=0.001), HbA1c (11.75 ± 2.18% vs. 9.83 ± 2.40%, p=0.004), and anti-GAD antibodies (168.08 ± 31.74 vs. 133.06 ± 16.94 pg/mL, p=0.001) seemed all increased in patients with CVB. According to the logistic regression, anti-GAD had a positive correlation with both FSG (r=0.53, p=0.002) and HbA1c (r=0.59, p=0.001).

Conclusion: Rising autoimmunity and reduced glycemic control were significantly linked to CVB infection. Their function as biomarkers for illness progression can be seen by the reality that elevated anti-GAD levels coincided with metabolic decline. The significance of combined serological and metabolic examinations in the management of type 1 diabetes is made apparent by the possibility that persistent CVB infection can both cause and exacerbate autoimmune diabetes.

Introduction

Genetic as well as environmental factors contribute to the chronic autoimmune disorder known as type 1 diabetes mellitus (T1DM) [1]. Enteroviruses, particularly Coxsackievirus B (CVB), are environmental factors that have been found extensively linked to the pathophysiology of type 1 diabetes [2,3]. Research indicates that cytolysis, molecular mimicry, and long-term inflammation are some of the ways that CVB infection encourages β-cell death [4,5]. Low-level chronic inflammation caused by a persistent CVB

infection can cause the pancreas to continually secrete antigens and produce autoantibodies [6]. Anti-glutamic acid decarboxylase (anti-GAD) is a key indicator of autoimmune diabetes that can be utilized for predicting the onset and course of the disease [7, 8]. Rapid β-cell reduction and low blood sugar levels have been associated with raised anti-GAD. Although research from all over the world has shown these correlations [9,11], little is known about the metabolic and serological correlates of CVB infection in children with type 1 diabetes in Iraq. The purpose of the present research was to examine how CVB affected Iraqi children's glycemic indices and anti-GAD levels in order to shed light on how viral persistence can exacerbate autoimmune and metabolic disorders.

Methods

The sample and study design: From November 2023 to February 2024, a case-control molecular study was carried out at Al-Diwaniyah Teaching Hospital in order to investigate into the relationship between Type 1 Diabetes Mellitus (T1DM) and Coxsackievirus B infection. In addition to age- and sex-matched, 56 children and adolescents (ages 3 to 15) with clinically and confirmed by testing type 1 diabetes were enrolled. Hygienic methods were used to obtain peripheral blood samples. Guardians and parents gave their informed consent.

RNA examination: 250 µL of peripheral blood has been collected using EDTA-containing tubes. Viral RNA has been isolated using the AccuZol Total RNA Extraction Kit (Bioneer, Korea). Both the concentration and purity were measured using the NanoDrop spectrophotometer. The reverse transcription procedure was performed using the M-MLV kit (Bioneer, Korea) and random hexamer primers. By targeting the conserved VP1 gene region, qRT-PCR was used for recognizing Coxsackievirus B (CVB) RNA. Vp1-F: 5′-GACACSATGCAAACAAGACA-3′ and Vp1-R: 5′-ATCCACACTAGAGGCAGTAC-3′ (amplicon size: 264 bp) were the primers used. 10 microliters of GoTaq® Probe qPCR Master Mix (Promega, USA), 5 microliters of cDNA, one microliter of each primer (10 pmol), one microliter of probe (20 pmol), and 2 microliters of nuclease-free water have been included in each 20 µL procedure.

Conditions of cycling: 2 minutes at 95 °C, 45 cycles of 15 s at 95 °C and 30 s at 60 °C, with the fluorescence reading at the conclusion of every single cycle.

Serological and metabolic testing: Traditional enzymatic testing were used to measure random blood sugar (RBS) and fasting serum glucose (FSG). The technique of high-performance liquid chromatography was used to measure HbA1c. Commercial ELISA kits (MyBiosource, USA) were used to measure the levels of anti-GAD antibodies.

SPSS v26.0 was utilized for statistical analysis. The independent t-tests were used for assessing continuous variables, which were presented as mean ± SD. Pearson correlation and logistic regression were used to evaluate correlations. A p-value of less than 0.05 was deemed significant.

Results

20 of the 56 children and adolescents diagnosed with T1DM had positive qRT-PCR results for CVB RNA (Figure 1) . Both metabolic and serological parameters showed distinct variations across the two sets.

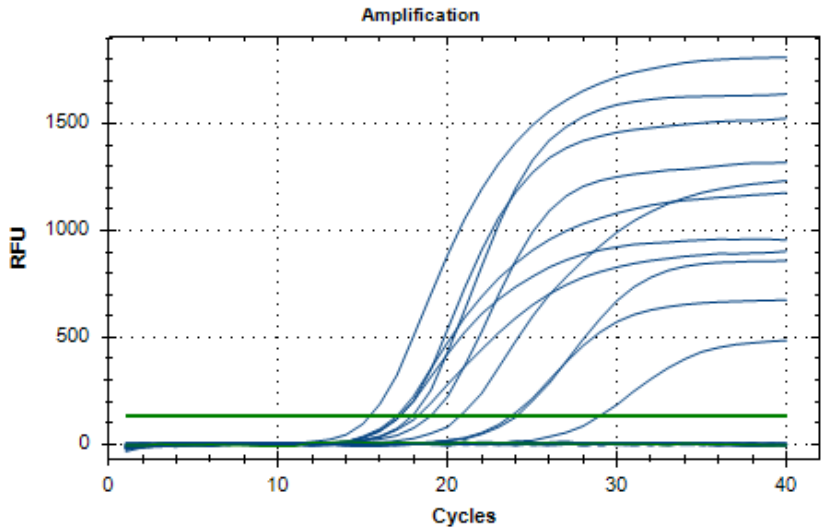

Figure 1: Coxsackievirus B infection identification in children and adolescents with type 1 diabetes using a real-time PCR amplification visualization.

Metabolic Profile: Pediatrics with CVB had a mean RBS of 268.9 ± 37.19 mg/dL, which was far greater than that of patients without CVB (223.47 ± 30.23 mg/dL, p=0.001). This suggests that reduced short-term glycemic control was linked to an acute viral infection.Pediatrics with CVB had a mean HbA1c of 11.75 ± 2.18%, while those without CVB had a mean HbA1c of 9.83 ± 2.40% (p=0.004). This means that chronic hyperglycemia and impaired long-term glucose regulation are caused by viral persistence (Table 1).

Metabolic factors	**CVB-Positive** ***n*** **=20**	**CVB-Negative** ***n*** **=36**	***P***
Random blood sugar (RBS) mg/dl			
Mean± SD	**268.9 ± 37.19**	**223.47 ± 30.23**	**0.001** † **S**
Range	135.0 – 460.0	86.40 – 423.0	
Glycated Hemoglobin (HbA1c %)			
Mean± SD	**11.75 ± 2.18**	**9.83 ± 2.40**	**0.004** † **S**
Range	8.70 -14.70	6.80-15.10	

Table 1: The comparison of metabolic factors (RBS and HbA1c) level according to results of Real-time PCR

Serological Identification: Anti-GAD (anti-glutamic acid decarboxylase) antibodies: Patients who tested positive for CVB had dosages that were noticeably higher (168.08 ± 31.74 pg/mL) than those who tested negative (133.06 ± 16.94 pg/mL, p=0.001). An increased autoimmune attack on β-cells in the context of a persistent viral infection is reflected in this increased activity. (Table 2) (Figure 2).

Parameters	***B1 genotype*** ***n* = 6**	**B3 *genotype*** ***n* =10**	**B4 *genotype*** ***n* =4**	***P***
anti-glutamic acid decarboxylase (GAD) level pg/ml				
Mean± SD	**163.66 ± 43.43^{A}**	**161.67 ± 34.24^{A}**	**186.25± 17.42^{B}**	**0.047** † **NS**
Range	111.45- 201.0	101.0 – 198.32	167.3 – 201.0	

Table 2: Serum anti-glutamic acid decarboxylase level in Patients with different *coxsackievirus B* genotypes.

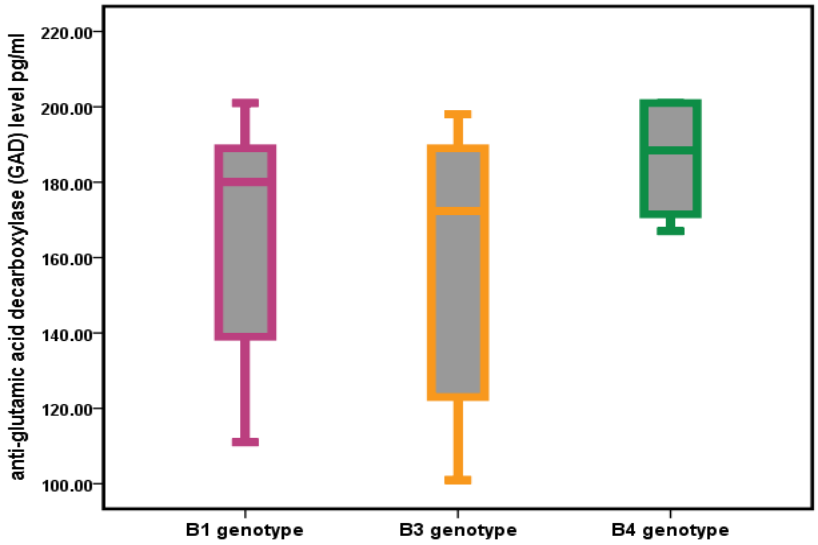

Figure (2): Serum anti- GAD levels in different *coxsackievirus B* genotypes

Anti-GAD showed a significant relationship with HbA1c (r=0.59, p=0.001) and with FSG (r=0.53, p=0.002), according to correlation analysis (Figure 3).

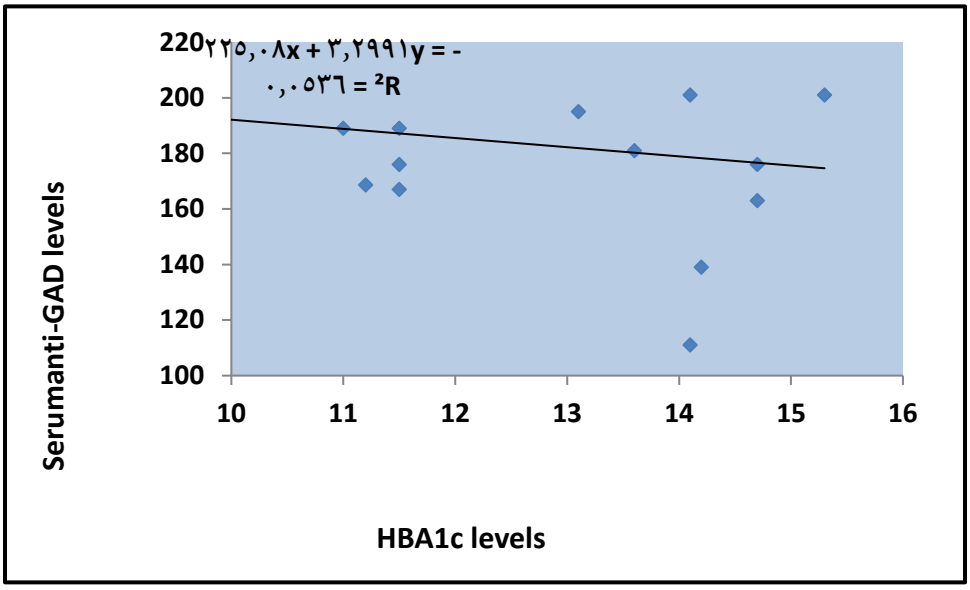

Figure (3): The Logestic scatter anti-GAD and HbA1c levels among patients.

Discussion

The current research demonstrates that in Iraqi children with type 1 diabetes, chronic CVB infection leads to both metabolic dysregulation and autoimmune stimulation. The negative impact of viral persistence on glycemic control is shown by the noticeably elevated HbA1c and glucose levels in patients with CVB [1,2]. There is data suggesting that CVB infection increases islet autoimmunity due to raised anti-GAD antibodies in infected children. The observed association between anti-GAD and both HbA1c and FSG indicates that the increasing rate of autoimmune β-cell degradation is accompanied by rising metabolic indices [3,4]. These results are consistent with research from other countries. Persistent CVB sustains the generation of autoantibody by maintaining severe pancreatic inflammation, as shown by Nekoua et al. [5] [6], who also connected CVB4 infection to elevated autoimmune antigens. In patients with diabetes, anti-GAD65 and CVB are significantly correlated [7]. The research's combination of metabolic and serological data reveals how persistent viral infection can both cause and enhance the risk of diabetes [8,9]. Psychologically, this indicate that tracking anti-GAD in addition to HbA1c may be a useful method to recognize children and adolescents who are at risk for developing the disease. Additionally, the evidence supports the investigation of immune-modulating or antiviral approaches as supplemental treatments for the control of diabetes [10,12]. The lack of long-term monitoring and the comparatively small study size are among the limitations. To establish correlation and ascertain the long-term effect of CVB on β-cell survival, potential multicenter experiments with bigger sample sizes and period monitoring are necessary.

Conclusion

In Iraqi children and adolescents suffering from type 1 diabetes, long-term CVB infection is substantially linked to reduced glycemic control and increased anti-GAD antibodies. The clinical significance of adding serological examinations into diabetes management strategies is underscored by both metabolic and serological correlates, which also show how viral persistence may boost β-cell autoimmunity

References

1. Lloyd R, et al. Enteroviruses and Type 1 Diabetes Mellitus. Annu Rev Med. 2022;73:483–499.
2. Dunne J, Richardson S, Atkinson M, et al. Rationale for enteroviral vaccination. Diabetologia. 2019;62:744–753.
3. Ahmed S, Ghanim A, Oday J. Correlation between Anti-GAD65 and CVB. Syst Rev Pharm. 2020;11(12):1620–1626.
4. Honkimaa A, Bryn K, Amir-Babak S, et al. Genetic adaptation of CVB1 in pancreatic cells. Microorganisms. 2020;8(11):1790.
5. Nekoua MP, Alidjinou EK, Hober D. Persistent Coxsackievirus B infection and T1DM. Nat Rev Endocrinol. 2022;18:503–516.
6. El-Senousy WM, Abdel-Moneim AS, Abdel-Latif MM, et al. CVB4 and T1DM. Food Environ Virol. 2018;10(1):89–98.
7. Meraj N, Rafiq M, Hussein H. Detection of CVB and TNF-α in T1DM. Int J Health Sci. 2022;6(S5):11565–11571.
8. Stone VM, Hankaniemi MM, Laitinen OH, et al. CVB vaccines prevent infection-accelerated diabetes. Diabetes. 2021;70(12):2871–2878.
9. Richardson SJ, Morgan NG. Enteroviral infections and T1DM: mechanisms of autoimmunity. Curr Diab Rep. 2018;18:36.
10. Yeung WC, Rawlinson WD, Craig ME. Enterovirus infection and T1DM: systematic review. BMJ. 2011;342:d35.
11. Krogvold L, Edwin B, Buanes T, et al. Detection of CVB in pancreatic islets of T1DM patients. Diabetologia. 2015;58:285–293.
12. Hyöty H. Viruses in T1DM: strong evidence for a link. Diabetologia. 2016;59:1141–1149.