Sawa Medical Journal

Reprint from

Vol.1 Issue.1 2025

Sawa Medical Journal

Prevalence and Genetic Diversity of Coxsackievirus B in Iraqi Pediatric Patients with Type 1 Diabetes Mellitus

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Abstract

The rate of infection and genetic diversity of Coxsackievirus B (CVB) in pediatric and teenagers with Type 1 Diabetes Mellitus (T1DM) were examined in a cross-sectional molecular study carried out at Al-Diwaniyah Teaching Hospital between November 2023 and February 2024. Finding current CVB genotypes and assessing their relationship with diabetes with young onset were the targets.

A total of 56 adolescents and children with T1DM participated in the study. Blood samples were used to obtain viral RNA, which was then transformed into complementary DNA (cDNA). To verify the presence of CVB RNA, the conserved part of the VP1 capsid gene was amplified using quantitative real-time PCR (qRT-PCR). Sanger sequencing and nested PCR were used to genotype the positive samples. SPSS v26.0 was used for statistical analysis.

Results revealed CVB RNA in 20/56 patients (35.7%). The mean age of CVB-positive cases (7.34 ± 3.42 years) was significantly lower than CVB-negative patients (10.78 ± 3.37 years). The 1–5-year age group had the highest positivity rate (45%). Infections were more common among females (60%) and rural residents (55%). VP1 sequencing identified three genotypes: CVB3 (50%), CVB1 (30%), and CVB4 (20%). Sequence comparison demonstrated high nucleotide homology (99.26–99.87%) between Iraqi isolates and reference strains in GenBank.

Conclusions: This study showed that several genotypes (B1, B3, and B4) common in Iraqi children with type 1 diabetes had a high prevalence of CVB infection. Younger children had higher infection rates, which lends trust to the idea that early viral exposure can cause autoimmunity.

Keywords: Iraq; T1DM; Coxsackievirus B; Genotypes; Prevalence

Introduction

Coxsackievirus B (CVB) is a non-enveloped, single-stranded RNA virus that belongs to the Picornaviridae family and Enterovirus B species. Many illnesses common to humans, from feverish sickness to more

serious conditions like myocarditis, meningitis, and pancreatitis, have been attributed to it [12]. Through direct beta-cell cytolysis, bystander activation, or molecular mimicry, many research investigations have linked chronic CVB infections to the autoimmune responses that contribute to Type 1 Diabetes Mellitus (T1DM) [8, 10]. The VP1 capsid protein is an important target for genotyping because of its wide range of genes and association with the serotype [3]. Molecular information on the distribution of genomes and frequency of CVB in Iraq's children with diabetes type 1 [2] remains insufficient throughout a great deal of global research. Therefore, the intention of this study was to use qRT-PCR to identify the prevalence of CVB in children with type 1 diabetes in Al-Diwaniyah, Iraq, and to perform VP1 gene sequencing and phylogenetic analysis to analyze the circulating genotypes.

Methods

Collecting Samples of Patients: This cross-sectional study included 56 children and adolescents with type 1 diabetes who were clinically diagnosed at the Al-Diwaniyah Teaching Hospital between November 2023 and February 2024. After obtaining informed consent, 250 µL of peripheral blood samples were collected in EDTA tubes for additional analysis.

RNA Extraction and cDNA Synthesis : Viral RNA was extracted from whole blood samples using the AccuZol Total RNA Extraction Kit (Bioneer, Korea) in compliance with the manufacturer's instructions. The concentration and purity of the extracted RNA were assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA). The extracted RNA was converted into complementary DNA (cDNA) using random hexamer primers and the M-MLV Reverse Transcriptase kit (Bioneer, Korea).

Quantitative Real-Time PCR (qRT-PCR): The presence of CVB RNA in the synthesized cDNA was tested using qRT-PCR and the GoTaq® qPCR Master Mix (Promega, USA). The assay targeted a conserved region of the VP1 capsid gene using particular primers (VP1-F: 5'-GACACSATGCAAACAAGACA-3'; VP1-R: 5'-ATCCACACTAGAGGCAGTAC-3'), which were based on the GenBank reference sequence GQ329737.1. The thermocycling conditions were as follows: 45 cycles of 95°C for 15 seconds and 60°C for 30 seconds were performed after initial denaturation at 95°C for 2 minutes.

Nested PCR and Sequencing: For genotyping, qRT-PCR-positive samples were put through a nested PCR process that amplified a longer segment of the VP1 gene using the GoTaq® G2 Green Master Mix (Promega, USA). The resulting amplicons were sent to Macrogen's Sanger sequencing facility in South Korea following purification.

Phylogenetic and Sequence Analysis: The obtained sequences have been analyzed and modified using ClustalW. MEGA software version 6.0 was used to create phylogenetic trees using the Maximum Composite Likelihood model and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Using bootstrap analysis with 1000 replicates, the phylogenetic groupings' robustness was measured. GenBank reference strains (e.g., GQ329737.1 for CVB1, GQ329767.1 for CVB3, and GQ329769.1 for CVB4) were compared to the generated sequences.

Statistical Analysis: All statistical analyses were carried out using SPSS version 26.0. Continuous variables were compared using the difference between samples t-test or one-way ANOVA, as appropriate,

and categorical variables were compared using the Chi-square test. A p-value of less than 0.05 was considered statistically significant. The data is displayed using the mean ± standard deviation (SD).

Results

CVB Infection Prevalence and Demographic Correlates: 20 (35.7%) of the 56 T1DM patients had positive qRT-PCR results for CVB RNA. Compared to the CVB-negative group (10.78 ± 3.37 years; p=0.023), the CVB-positive group was significantly younger (7.34 ± 3.42 years). According to table (1), a greater percentage of positive cases were discovered in the 1–5 year age group (45%), among females (60%), and among those living in rural areas (55%).

Table (1): The comparison of mean age, Sex and residency distribution according to results of Real-time PCR

Characteristic	Total *n* = 56	CVB-Positive *n* =20	CVB-Negative *n* =36	*p*
Age (years)				
Mean ±SD	9.51 ± 2.19	7.34 ±3.42	10.78 ±3.37	0.023 † S
Range	3 -15	3-14	3-15	
1-5 year, *n* (%)	12 (21.4%)	9 (45.0%)	3 (8.3%)	0.005 ¥ S
6-10 year, *n* (%)	20 (35.7 %)	6 (30.0%)	14 (38.9%)	
11-15 year, *n* (%)	24 (42.9%)	5 (25.0%)	19 (52.8%)	
Sex				
Male, *n* (%)	27 (48.2%)	8 (40.0%)	19 (52.8%)	0.359 ¥ NS
Female, *n* (%)	29 (51.8%)	12 (60.0 %)	17 (47.2%)	
Residency				
Urban, *n* (%)	24 (42.9%)	9 (45.0 %)	15 (41.7 %)	0.809 ¥ NS
Rural, *n* (%)	32 (57.1%)	11 (55.0 %)	21 (58.3 %)	

Phylogenetic Analysis and Genotypic Distribution: Sequencing of the VP1 gene from all 20 positive isolates identified three genotypes: CVB3 was the most prevalent (n=10, 50%), followed by CVB1 (n=6, 30%) and CVB4 (n=4, 20%) (Table 2). BLAST analysis revealed very high sequence identity (99.26% to 99.87%) between the local Iraqi isolates and their corresponding GenBank reference sequences. Phylogenetic tree analysis confirmed the genotyping results, with local isolates clustering robustly within distinct clades corresponding to CVB1, CVB3, and CVB4 genotypes alongside their international

reference strains (Figure 1). The multiple sequence alignment showed a highly conserved VP1 sequence with sporadic point mutations distinguishing the isolates .

Table (2): Frequency distribution of *coxsackievirus B* according *B genotypes.*

Genotypes	NO (%)	P value
B1, *n* (%)	6 (30.0%)	0.247 ¥ NS
B3, *n* (%)	10 (50.0 %)	
B4, *n* (%)	4 (20.0%)	

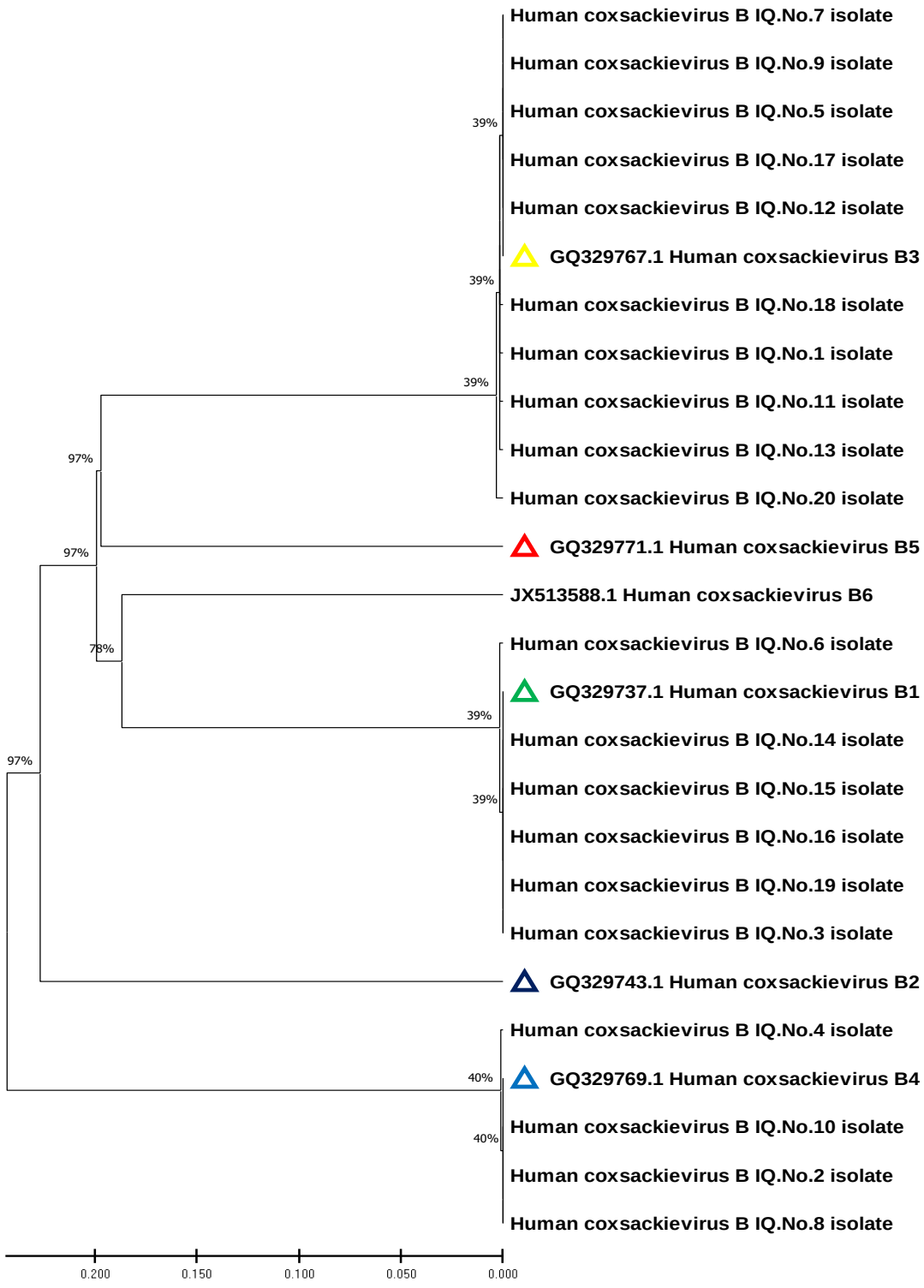

Figure (1): The capsid protein VP1 gene partial sequence in local Coxsackievirus B isolates used for genotyping analysis was based on phylogenetic tree analysis.

Discussion

20 of the patients (35.7%) out of the 56 T1DM children and adolescents who participated in the study tested positive for CVB RNA using qRT-PCR. According to this prevalence percentage, nearly a third of the pediatric diabetic study subjects had a CVB infection, which is in line with prior studies that strongly links CVB to autoimmune diabetes [1,10].

With a mean age of 7.34 ± 3.42 years, the CVB-positive group was much younger than the CVB-negative group, which had a mean age of 10.78 ± 3.37 years ($p = 0.023$). Stratified by age group, 45% of positive cases fell within the 1–5 year age range, while only 8.3% of negative cases did the same. In contrast to the CVB-negative cohort, which had 38.9% and 52.8% of positives, respectively, 30% of positives were in the 6–10 year range and 25% in the 11–15 year group. According to these outcomes, the most severe risk period for CVB infection is early childhood, which confirms the idea that early viral exposure may play a role in the development of autoimmunity [6,9]

This sex-based difference was not of statistical significance ($p = 0.359$), considering having been found that 60% of CVB-positive patients were female and 40% were male. Similarly, there was no significant difference in infection rates by residence: 55% of positive cases came from rural areas, compared to 45% from urban areas ($p = 0.809$). These non-significant trends might be a result of lifestyle and environmental factors that have been linked to viral transmission, including being exposed to water that has not been filtered, access to health care, and hygiene [5].

Genotyping of the VP1 gene from positive samples revealed three circulating CVB genotypes.Even though CVB3 predominated in this Iraqi population, nearby studies have shown multiple significant types, such as CVB4 in Egypt [5] and in local Iraqi studies [7]. The seasonal and geographic variations in CVB epidemiology is indicated by these disparities. Nucleotide homology between the Iraqi isolates and their corresponding reference strains from GenBank ranged from 99.26% to 99.87%, indicating a very high degree of genetic similarity, according the sequencing analysis.

With no indication of distinct local variations, phylogenetic clustering verified that the isolates belonged to the global CVB1, CVB3, and CVB4 lineages. This clearly shows that the Iraqi strains are a part of the global spread of enteroviruses [8] and the wider world transmission of CVB.

All things considered, the study shows that CVB is very common in Iraqi children who have type 1 diabetes, with adolescents showing the greatest relationship. The genetic variation of CVB in this population has been demonstrated by its discovery of several common genotypes (B1, B3, and B4), and the lineages' persistence and globally transmission are supported by their strong similarities with global strains.

conclusion

This study found that Coxsackievirus B (CVB) was extremely common in Iraqi children with type 1 diabetes, with multiple genotypes (B1, B3, and B4) co-circulating. Increased infection rates were seen in younger children, which supports the theory that early viral exposure can trigger autoimmunity. High genetic homology with global strains indicates international circulation. These findings highlight the

need for continuous monitoring and preventative actions, as well as the probable role of CVB in the pathophysiology of type 1 diabetes in Iraq.

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