Original Article

Innovation in the Diagnosis of Coxsackie B Virus

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Abstract

Background and Objective: Coxsackie B virus (CVB) was first isolated in the late 1940s in Coxsackie, New York, and is now known to be widespread worldwide. It particle is small, nonenveloped, and contains a positive single-stranded RNA genome, primarily causes viral myocarditis through direct myocardial injury and immune-mediated damage, potentially progressing to dilated cardiomyopathy. This study aimed to detect the prevalence of *Coxsackie B virus* in heart patients and healthy control and to detect *B3* gene by real-time polymerase chain reaction. **Material and Method:** The study conducted in general Cardiac Center Erbil/Iraq. A total (140) blood sample was collected, (90) from heart patients and (50) from healthy control in order to estimate prevalence of Coxsackie B virus antibodies IgG and IgM serological by using ELISA and molecular detection of *B3* gene by real-time polymerase chain reaction. **Result:** The total seropositivity of Coxsackie B virus IgG and IgM was (3.3,5.5) for heart patients and (4%) for healthy control serologically, while (100%) were positive for molecular detection of *Coxsackie B3* gene. **Conclusion:** Its conclude that, *there was no significant association between Coxsackie B virus antibodies (IgG,IgM) and myocarditis ;positivity rate were low and similar in both patients and control*. The *B3* gene is a sensitive marker for molecular detection of Coxsackie virus, particularly in heart patients.

Keywords: Coxsackie Virus, Myocarditis, RT-PCR, B3 Gene.

Introduction

Coxsackie B virus, part of the enterovirus family, is a non-enveloped RNA virus with a strong tendency to target heart tissue. It is a primary cause of viral myocarditis, a condition involving inflammation of the heart muscle that can lead to acute heart failure, irregular heartbeats, or long-term dilated cardiomyopathy.[1] The coxsackie B virus typically spreads through the fecal-oral route, though it can also transmit via respiratory droplets, especially in crowded or unhygienic environments. Once it reaches the bloodstream, Coxsackie B virus targets the myocardium, directly infecting heart muscle cells. As the virus replicates inside these cells, it causes cell destruction and tissue damage, leads to the inflammation.[2] The symptoms caused by Coxsackie B virus infections often reveal as myocarditis, with signs like chest pain, tiredness, difficulty breathing, and, in extreme cases, sudden cardiac death, particularly in young, otherwise healthy people.[3,4] The viral infection may initially present with flu-like symptoms, indicating a complication with other viruses before its systemic spread before it focuses on the heart.

Coxsackie B virus is particularly notable for their distinct effects on the heart, contributing to serious illness and death worldwide. Understanding how this pathogen affects the heart is important for improving diagnosis, treatment, and prevention efforts especially in unsuitable environments.[5,6] The objectives of this study were to detect the prevalence of *Coxsackie B virus* serologically and molecularly in heart disease patients.

Material and Method

Study Population

The study took place in Erbil Cardiac center, Erbil, Iraq. On total 140 patients and healthy control (HC) group with age group 21-65 years from September 2024 to March 2025.

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Sample Collection

5 milliliters of venous blood samples were collected from patients and healthy individuals for comparison under sterile conditions after vein identification and venipuncture were placed in standard tubes containing a gel substance and centrifuged at 3000 rpm for 10 minutes to separate the serum. The serum was then transferred to Eppendorf tubes using a micropipette and stored at -80°C in deep freezing, to detection the Coxsackie B virus. 2 ml of collected blood were put into EDTA tubes and delivered to the laboratory for for RNA extraction in order to use in RT-PCR detection Sero type(B3) responsible for heart patients.

Enzyme- linked Immunosorbent Assay (ELISA)

This test was carried out to detect Coxsackie B virus is designed for the qualitative determination of Coxsackie Virus B-specific (IgG, IgM) antibodies in human serum, plasma, culture media, or other biological fluids. According to protocol provided by the manufacture company of the kit.

RNA Extraction and the Molecular Detection of Cox. Virus B3 Gene by Real-time PCR Techniques

The all positive samples for *CVB*[7] abs (IgM and IgG) from both patients and healthy group were subjected to *CVB3* gene molecular detection. RNA extraction were performed on 10 samples and RNA extraction were carried out using extraction easy pure RNA or DNA KIT In order to carry out PCR reaction test , the PCR mixture were prepared by adding all the essential components according to *EasyScript*® One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen, biotech. AE311-02). as listed in table (1).

Table 1:

Component	Volume for each Sample in Ml
RT/RI Enzyme Mix	50
gDNA Remover	50
Reaction Mix	500
Random Primer	50
Anchored Oligo	50
RNase-free Water	500

Synthesis the c DNA from m RNA in Table 2

Table 2:

Component	Volume Reaction in Ml
mRNA	6
Anchored Oligo	1
Random Primer	1
Reaction Mix	10
RT/RI Enzyme Mix	1
gDNA Remover	1
RNase-free Water	3
Total volume	23

Thermal Cycler Steps for cDNA Reverse Transcription Conditions in Table 3

Table 3:

	Step 1	Step 2	Step 3
Temperature	25 ºC	42°C	85 ºC
Time	10 min	15 min	5 Sec.

Statistical Analysis

The data obtained from this study analyzed using Statistical Package for the Social Sciences (SPSS) program (one way ANOVA and Chi-square test). P- value was considered significant when it's < 0.05.

Result

The serum of 140 participants in this study were screened for presence of *CVB* Abs (IgM and IgG) and the total seropositivity for CVB was (3.3,5.5) for heart patients as shown in table (4). No statistically significant association was observed between CBV seropositivity (IgM and IgG) and myocarditis diagnosis in this cohort. The low IgM positivity rate (3.3% in patients, 0% in controls) and comparable IgG rates (5.5% vs. 4%) suggest no substantial virological link to myocarditis in this sample.

Table 4: Positive Infections among CBV Infection and Control Group.

CBV	Myocarditis Patients		Control Group		P. value
	No=90	%	No=50	%	
IgM+	3	3.3%	0	0.0%	0.537
IgM-	87	96%	50	100%	NS
IgG+	5	5.5%	2	4%	1.0
IgG-	85	94%	48	96%	NS

*NS mean non significant

As appear in table (5), The data indicate that Coxsackie B virus (CVB) seropositivity is slightly higher among male participants (11.6%) compared to female participants (8.1%). This difference is primarily due to the presence of IgM antibodies, which were detected only in males (11.6%), suggesting a higher rate of recent and chronic CBV infection among male subjects. In contrast, IgG seropositivity—indicative of past exposure—was observed in both genders (8.8%), showing a marginally higher rate as shown in Table 5.

Table 5: Gender Variation in CVB Infections among Participants.

Gender	Positive CBV Infections				P value
	N.	+ IgM	+ IgG	%	
Male	43	3	2	11.6 %	0.71 NS
Female	37	0	3	8.1 %	
Total	90	3	5	8.8 %	

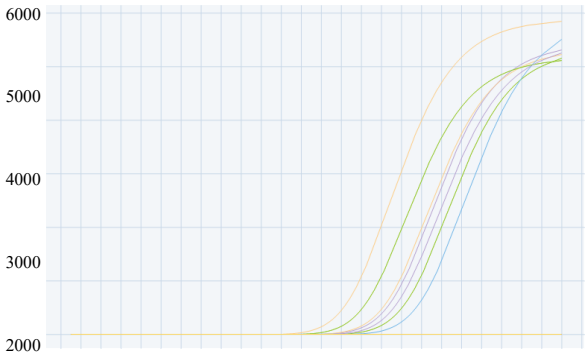

Figure 1: The Amplification Curve of RT-PCR to Detect *B3* Gene of *Coxsackie Virus.*

With regards to RT-PCR results, from total 7 positive samples to *CVB3* (3 were IgM and 9 were IgG in heart patient) were detect the presence of *CVB3 gene* (samples that contain *B3* gene of *CVB* showed sigmoid amplification curve which mean positive sample for *CVB3.* (Figure 1)

Discussion

Several previous studies have explored This finding aligns with case-control study, which found no significant difference in CBV IgM between myocarditis patients and controls, with only IgM positivity in patients versus controls. A prior study also observed IgM antibodies in 27% of myocarditis/pericarditis patients versus 8% in controls, suggesting IgM is detectable in a minority of cases.[5] Whereas noted CBV involvement in 25–40% of myocarditis cases, yet IgM was not consistently detected even in PCR-confirmed infections.[6] The factors contributing to low IgM may be that report the test timing as IgM antibodies emerge early (days 5–7 post-infection) and wane within weeks. Testing during convalescence may yield false negatives.[8]

In current study, this may be due to traditional serology (e.g., ELISA) may miss low-titer antibodies. Studies using advanced methods (e.g., pathogen-targeted NGS) report higher CBV detection than serology alone.[7] Regarding IgG antibodies, which represent prior exposure or long-term immune memory, both groups showed nearly identical positivity rates 5.5% in heart disease patients (4 out of 90) and 4% in the control group (2 out of 50). This statistical discrepancy may reflect variation in antibody persistence or immune memory functionality, particularly in individuals with chronic conditions such as cardiovascular disease.[9] The shared IgG positivity across both groups implies that prior exposure to Coxsackie B virus may be common in the general population, reflecting the virus's endemic presence in the community.[10]

Also their study demonstrated that since IgM antibodies are produced early during infection and decline as the infection resolves, the stronger and more rapid immune response in females may mean that the window during which IgM is detectable is shorter. In contrast, males who often have a less robust immune response may experience a longer persistence of IgM, making it more likely to be detected at the time of testing.[11]j®C Males may have higher rates of IgM positivity for CBV because their immune response is typically less robust and slower to clear the virus, resulting in a more extended period during which IgM antibodies are detectable. Females, with a stronger and faster immune response, may clear the infection more quickly, leading to a shorter window for IgM detection.[12] It is worth to take in consideration, Studies in both humans and animal models show that males are often more susceptible to viral infections and may have prolonged or less effective immune responses, leading to extended periods of IgM positivity.[9] The detection of Coxsackie virus required a combination of serological and molecular techniques.[13]

So serological methods such as ELISA and there are several types of molecular diagnostic method such conventional PCR, nested PCR, loop- mediated isothermal amplification (LAMP), multiplex PCR, and RT.PCR. Among these types, RT- PCR technique has high specificity, reliability and efficiency and low rate of contamination with no need to gel electrophoresis to visualize the results.[14] The primary purpose of laboratory diagnostics is to identify and stop major epidemics.[15]

The selection of appropriate biological samples is crucial for the molecular diagnosis of viral diseases, as sample type significantly influences the accuracy, sensitivity, and reliability of diagnostic outcomes[16] Over and above to that, RT-PCR able to detect small quantity of RNA. [17] The use of amplification of DNA techniques, such as polymerase chain reaction (PCR) has been important to detect these viral infections. Serology is also used to identify The IgM and IgG. In addition, the immunoassays are the most frequently used serological assays.[18,19] In this study, *B3* gene was used as a target gene for detection as it found in Coxsackie virus B From total 10 samples positive for IgM and IgG by ELISA, only 8 sample of them were detected the presence of *CVB3* gene in their samples. All 8 positive sample for *B3* gene were in myocarditis patients.

Conclusion

- It can be concluded that the molecular and serological detection of CVB in myocarditis patient play an essential role to know there was no significant association between Coxsackievirus B antibodies (IgM, IgG) and myocarditis; positivity rates were low and similar in both patients and controls.
- Recent CVB infection (IgM positivity) was slightly higher in males, but this difference was not statistically significant.

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