

# Coxsackie virus B detection of some Heart disease - Cases with rheumatic arthritis

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## ABSTRACT

**Aim:** To highlight the relationship between heart disease and viral Coxsackie virus B infection. The study included heart disease patients who have confirmed cases of heart disease and entered the open-heart center in Sadr Medical City.

**Methods:** Patients were divided into three categories: patients with myocarditis, patients with other heart disease and patients with rheumatic arthritis. 224 blood samples were taken from these patients' involved 87 males and 137 females to investigate rheumatoid arthritis as well as to investigate viral infections by serological and molecular technique in order to detect antiviral antibodies and viral nucleic acid.

**Results:** Out of all studied samples, there were 37.5% positive results for viral infection among heart disease patients, 47.6% of them those with rheumatic arthritis and 52.4% without rheumatic arthritis.

**Conclusion:** The recent study indicated that 80% of infected patients with myocarditis and the remaining 20% with other heart diseases.

**Keywords:** Coxsackieviruses, Myocarditis, Heart disease, Rheumatic arthritis, ELISA, RT-PCR.

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## INTRODUCTION

Coxsackieviruses is a member of Picornaviridae family, small RNA-containing viruses, 25–30 nm in diameter. They are divided into two groups, A and B. There are 23 serotypes of coxsackie A viruses and at least six serotypes of coxsackie B virus<sup>1</sup>. Those from the group B are associated with more severe illness.

Human, spread by direct contact with nasal and throat secretions, faecal–oral route and inhalation of infected aerosols. It has worldwide distribution, with increased frequency occurring in warm months in temperate climates.

They can remain viable for many years at extremely low temperatures (between minus 20 °C and since the 1960s, it has been suggested that group B coxsackieviruses are the most frequent viral etiological agent associated with heart diseases including myocarditis, pericarditis and endocarditis<sup>2,3</sup>, causing more than 50% of all cases of viral myocarditis<sup>4</sup>.

The presence of heart-specific autoantibodies in the sera of some patients with coxsackievirus B3-induced myocarditis has suggested that autoimmunity is a sequela of viral myocarditis<sup>5</sup>. Potentially, autoimmunity can develop in genetically predisposed individuals whenever damage is done to the cardiac tissue. Coxsackievirus has been implicated in cases of arthritis and arthralgias<sup>6,7</sup>. There are a few reports suggesting an association of coxsackievirus with rheumatic fever<sup>8,9,10</sup>.

Myocarditis is a non-familial form of heart muscle disease<sup>11,12</sup>, defined as an inflammation of the heart muscle, and identified by immunohistological, clinical or histopathologic criteria<sup>12,13,14</sup>. Previous studies have demonstrated that viral myocarditis and the affiliate

inflammatory response can be stated as basic determinants of the degree of virus-associated myocardial damage<sup>15</sup>. Viral myocarditis is regarded as a moderate disease, though it sometimes causes irreversible myoc

Many different viruses have been considered as the agent of viral myocarditis, including enteroviruses, cytomegalovirus, adenovirus, parvovirus B19 and hepatitis C virus<sup>17</sup>. Among the normal infectious agents, the group B coxsackie viruses are important pathogens of viral myocarditis, which accounts for more than 50% of viral myocarditis cases<sup>16</sup>. The group B coxsackieviruses consists of six serotypes, which are members of the Enterovirus genus within the Picornaviridae family<sup>18,19,20,21</sup>.

Myocarditis is clinically and pathologically defined as an inflammation of the heart muscle<sup>22</sup>. The term myocarditis was first used in the early 19th century to describe myocardial diseases not associated with valvular abnormalities<sup>23</sup>, but only in the second half of the 20th century was interest in inflammatory myocardial diseases renewed<sup>24</sup>. A number of patients with acute viral myocarditis may develop dilated cardiomyopathy as a complication<sup>25,26</sup>. Patients who have suffered from a heart attack may develop pericarditis over the subsequent days or weeks<sup>22,27,28</sup>.

## MATERIALS AND METHODS

**Study design:** The study was designed according to the research method (across sectional study), 224 blood samples were obtained randomly from heart disease patients and have medical records and have been diagnosed with heart disease for sure who entered the

open heart center in Sadr Medical City in Al- Najaf governorate.

These patients were divided into two groups: Patients with myocarditis and patients with other heart disease, also classified into patients with rheumatoid arthritis and patients without rheumatoid arthritis by using Rapid RA Kit.

All samples were taken to detect viral infection in all study groups anti-viral antibodies using ELISA, and finally to diagnose viral RNA in the whole blood sample using Real Time PCR.

**Serological tests:** Rapid RA Kit Antibodies against cyclic citrullinated peptides (CCP) - FAVOGEN (Korea). Human Antistreptolysin O ELISA Kit (Quantitative Sandwich ELISA kit). IBL-America Coxsackie virus IgM ELISA Coxsackie virus IgM Order (no.: IB05041), Coxsackie virus IgG Order (no. IB05040).

**Molecular technique:** Reverse transcriptase-polymerase chain reaction (RT-PCR) (14-n). The viral RNA was extracted from blood samples using the TRIzol® Plus RNA Purification kit (Invitrogen Life Technologies) according to the manufacturer's instructions.

DNase treatment during RNA purification was adopted using the PureLink™ DNase (Invitrogen Life Technologies) in order to obtain DNA-free total RNA.

One-step RT-PCR for the detection of viral RNA was performed with primers directed to the conserved sequences in the 5'-UTR of genome. A fragment of 155 bp of the extracted RNA was amplified by one-step RT-PCR (Invitrogen SuperScript™ One-Step RT-PCR with Platinum® Taq) using 006 and 007 primers (29). The RT-PCR was performed on a mixture (RT/Platinum® Taq; Invitrogen Life Technologies)

The reaction was conducted with an initial reverse transcription step at 42°C for 30 min, followed by PCR activation at 94°C for 5 min, 30 amplification cycles (94°C, 30 sec; 42°C, 1 min; 72°C, 2 min) and a final 10-min extension at 72°C in an Eppendorf Mastercycler Thermal Cycler. The PCR products were run on a 2% agarose gel stained with ethidium bromide and visualized under UV light.

**Statistical Analysis:** The Chi-square test was applied to determine the statistical significance of the data. P value of <0.05 was considered significant.

## RESULTS

Heart diseases cases according to age groups and gender. Heart disease patients were divided into 87 (39%) males and 137 (61%) females, and according to age groups, the age groups (20-44) and (45-64) were the highest number of cases. (Table 1).

**Detection of viral infection in heart diseases patients according to age groups:** The investigation of viral infections in heart diseases patients according to age groups revealed that out of a total 224 cases there were 84 (38%) gave positive result, while 140 (62%) was negative result of viral examination. In addition, the results showed that the age groups (20 – 44) and (45 – 64) have the highest percentage of infected cases (Table 2).

**Types of heart diseases of infected cases:** The infected cases classified into two types: myocarditis and other heart diseases, the study showed that out of a total number of

infected cases, patients with myocarditis were 67 (80%) while 17 (20%) with other heart diseases (Table 3).

**Rheumatoid arthritis cases of infected heart diseases patients:** Table 4 showed that infected patients with Coxsackieviruses were divided into groups by using serological tests into 40 (48%) patients with rheumatoid arthritis and other 44 (52%) without rheumatoid arthritis.

Table 1: Heart diseases cases according to age groups and gender

Age groups	n	Male	Female
1 – 4	14	4	10
5 - 9	8	4	4
10 - 14	4	1	3
15 - 19	14	4	10
20 - 44	57	24	33
45 - 64	82	32	50
> 65	39	15	24
Total	224	87 (39%)	137 (61%)

Table 2: Detection of viral infection in heart diseases patients according to age groups

Age groups	n	Coxsackieviruses Detection +	Coxsackieviruses Detection -
1 – 4	14	1	13
5 - 9	8	1	9
10 - 14	4	0	4
15 - 19	14	7	7
20 - 44	57	26	31
45 - 64	82	35	47
> 65	39	21	18
Total	224	84 (38%)	140 (62%)

Table 3: Types of heart diseases of infected cases

Age groups	Coxsackieviruses Detection +	Myocarditis	Other cardiac diseases
1 – 4	1	0	1
5 - 9	1	0	0
10 - 14	0	0	0
15 - 19	7	6	1
20 - 44	26	23	3
45 - 64	35	28	7
> 65	21	16	5
Total	84	67 (80%)	17(20%)

Table 4: Rheumatoid arthritis cases of infected heart diseases patients

Age groups	Coxsackieviruses Detection +	Non streptococcal rheumatoid arthritis	Without rheumatoid arthritis
1 – 4	1	0	1
5 – 9	1	0	1
10 – 14	0	0	0
15 – 19	7	5	2
20 – 44	26	9	17
45 – 64	35	16	19
> 65	21	10	11
Total	84	40 (48%)	44 ( 52%)

By investigating the virus in all health cases, which were considered as a control group that were included in the study, it was revealed in the results and through Table No. 5 that the number of cases infected with the virus is six cases 4% of the total number of healthy people, while the rest were negative.

Table 5: Detection of viral infection in healthy individuals (control group) according to age groups

Age groups	n	Coxsackieviruses Detection +	Coxsackieviruses Detection -
1 – 4	13	0	13
5 - 9	10	0	10
10 - 14	9	0	9
15 - 19	13	0	13
20 - 44	33	2	31
45 - 64	47	2	45
> 65	25	2	24
Total	150	6 ( 4%)	144 ( 96%)

**DISCUSSION:**

The present study, conducted in the open hear center in al-Najaf governorate, aimed to investigate the presence of Coxsackieviruses infection (Antiviral Ab and genomic RNA) in blood in hospitalized patients suffering from myocardial and other heart diseases.

Presence of viral infection was identified in 84 cases (38%) using ELSA and RT-PCR technique. Having demonstrated that virus is often involved in two forms of infectious heart diseases, sequencing was adopted to confirm the involvement of these viruses. Epidemiological studies linking a suspected viral outbreak with water are difficult because limited waterborne viral outbreaks usually occur at distance from the original source of contamination<sup>30</sup>.

When a comparison is made in our study between heart patients and healthy people, it was found that among the total number of healthy people, only 4% of them are infected with the virus, and this means that healthy people who do not suffer from heart disease may be exposed to the virus, but at very rare rates, unlike what is found in Cases of heart patients, and this indicates that there may be a close relationship between heart patients and viral infection

However<sup>31</sup> studied 296 children with symptoms typical of an enteroviral infection, and 679 controls with no symptoms. Viruses were isolated from 287 cases, group A coxsackieviruses were isolated from 45 of these and group B coxsackievirus from 29. A history of swimming was obtained from all cases and controls. It was concluded that children from whom an enterovirus was isolated were more likely to have swum at a beach than controls. Those who only swum in a swimming pool were not at increased risk. Case children from whom no virus was isolated did not differ from healthy controls.

In both groups of healthy and sick, the older age groups are the ones that are exposed to the viral infection than the younger ones, and this may indicate the possibility of exposure to infection with advancing age or the development of a person's disease

In May 1992, a 20-year old man developed nausea following a surfing outing in Malibu. His symptoms grew

progressively worse and coxsackie B virus was isolated from him. He subsequently died from damage to his heart, caused by the virus. Although it was not proved that the virus was contracted whilst surfing, it was thought that this was the case<sup>32</sup>.

The present epidemiological study has provided some new results of marked interest. Age, gender and seasonal variation appear to be important factors affecting the CV-B prevalence in human infectious heart diseases. Although enterovirus infections in Tunisia generally reach a peak in autumn <sup>(33)</sup>, this seasonal variation was also found to progress until winter, as demonstrated in the present study.

According to age, CV-B specifically affects young male adults, possibly because they have a more intense and active lifestyle. Finally, males appear to be more susceptible to these infections, which may raise the hypothesis of different hormone secretions.

L. Andréoletti, P. Wattré 1999<sup>34</sup> revealed that Persistent cardiac infection induces a Th1 immunological response, auto-immune mechanisms as molecular mimicry, autoreactive T-cells and auto-antibodies directed against myocytes antigenic structures. Moreover, the development of endomyocardial viral persistence could be linked to unknown mechanisms allowing coxsackie B viruses to avoid immunologic surveillance.

To the best of our knowledge, the present study is the first report of the epidemiology of CV-B heart infections in Iraq. These viruses are associated with various diseases including heart diseases and may cause major problems of human health.

**CONCLUSIONS:**

- 1- The present study suggests that the heart diseases may be highly associated with CV-B infection
- 2- Myocarditis involved the largest infected cases with Coxsackieviruses than other heart diseases cases.
- 3- The heart diseases cases with rheumatic arthritis have high percentage if viral infection than cases without RA.

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