

Seroprevalance of Coxsackie virus B among patients with Heart Diseases in Khartoum Cardiac Center

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Abstract:

Background: *Coxsackie B viruses (CVB) are estimated to be responsible for at least 50% of the cases of infection caused heart diseases. For reasons yet unknown, the Coxsackie B virus is directly linked to and responsible for many unexplained and sudden heart related events that can strike any healthy adults at a time with unknown symptoms of current heart disease.*

Methods: *This is a cross sectional study in which 45 heart disease patients and 45 healthy individuals, were enrolled their age ranged between 14-80 years old with mean 47 years old, conducted in two cardiac center in Khartoum, State, Sudan, during February to May 2015, to detect CVB IgG seroprevalance using commercially available enzyme-linked immunosorbent assay kit. Generated data were analyzed by using SPSS program*

Results: *out of the total, 45 patient with heart disease, 29 (64.4%)were males, and 16(35.6%) females, the result showed that 31(68.9%) were positive for CVB IgG, however among 45 healthy individuals (control group), 35(77.8%) were males and 10(22,2%) females, 19(42.2%)showed positive result. The statistical analysis*

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showed there was insignificant correlation between seropositivity of CVB and age, gender, type of heart diseases and duration of it.

Conclusion: *large scale studies in different settings and studies in Sudan are required.*

Key words: Seroprevalence, Coxsackie virus B, IgG, Heart diseases, Khartoum, Sudan.

Introduction:

Coxsackie refers to a collection of closely related viruses classified among the enteroviruses, namely those that cause infection after being taken in orally with contaminated food or water and then multiply in the intestines. The coxsackie viruses were named after the town Coxsackie, New York. A strain of this virus was discovered there during the investigation of an epidemic that occurred in 1948 alongside a polio epidemic (polio being another enterovirus). The coxsackie viruses are divided into two major subgroups, labeled A and B. There are 23 known coxsackie A viruses that usually cause only enteric diseases, and 6 known coxsackie B viruses, which are the ones of greatest concern because of their ability to cause serious diseases beyond the intestinal tract. Coxsackie B3 has been found to be one of the main causes of certain debilitating or life-threatening diseases, such as viral myocarditis.¹

The various members of the Coxsackie B group were discovered almost entirely in the United States, appearing originally in Connecticut, Ohio, New York, and Kentucky, although a sixth member of the group has been found in the Philippines(?). That said, all six serotypes have a Symptoms of infection with viruses in the Coxsackie B grouping include fever, headache, sore throat, gastrointestinal distress, as well as chest and muscle pain. Group B coxsackie viruses tend to infect the heart, pleura, pancreas, and liver, causing

pleurodynia, myocarditis, pericarditis. This presentation is known as pleurodynia or Bornholm disease in many areas. Sufferers of chest pain should see a doctor immediately—in some cases, viruses in the Coxsackie B family progress to myocarditis or pericarditis, which can result in permanent heart damage or death. Coxsackie B virus infection may also induce aseptic meningitis. As a group, they are the most common cause of unexpected sudden death, and may account for up to 50% of such cases (3). The incubation period for the Coxsackie B viruses ranges from 2 to 6 days, and illness may last for up to two weeks, but may resolve as quickly as two days. Infection usually occurs between the months of June and October in temperate Northern Hemisphere regions global distribution and is a relatively common cause of gastrointestinal upset. Enterovirus infection is diagnosed mainly via serological tests such as ELISA(4) and from cell culture(2). Because the same level and type of care is given regardless of type of Coxsackie B infection, associated disease of coxsackie virus If acquired in the first trimester of pregnancy Coxsackie viruses can cause spontaneous abortion. Maternal Coxsackie virus infections have also been associated with type 1 diabetes in the off spring. Maternal Coxsackie virus B infection has been associated to an increase in fetal cardiac abnormalities. Coxsackie viruses A and B can cross the placenta and cause stillbirth by villous necrosis and a variety of other mean[3]. Coxsackie virus infection has also been linked with chronic fatigue syndrome (CFS), also referred to as myalgic encephalitis (ME).(5)

Coxsackievirus group B type 3 (CVB3) is an important cause of viral myocarditis. The infiltration of mononuclear cells into the myocardial tissue is one of the key events in viral myocarditis. Immediately after CVB3 infects the heart, the expression of chemokine(s) by infected myocardial cells may be the first trigger for inflammatory infiltration and immune response. (6)

Materials and Methods:

Design: This is across sectional study included heart disease patients and healthy individuals (control group) aged between 14-80 years old with mean 47 years old conducted in two cardiac center in khartoum, State, Sudan, during February to May 2015.

CVB serum marker, was detected using commercially available enzyme-linked immunosorbent assay. The data was collected by structured questionnaire. Ethical approval was taken from Al Neelain University research ethical board and with consent was obtained from patients verbally.

Experimental work: Serum specimens were collected from known heart disease patients, and healthy individuals and screened for CVB IgG antibodies using enzyme linked immune sorbent assay (ELISA) (GmbH, Würzburg, Germany) technique at research laboratory \AL Neelain University.

Collection of specimens and processing: Three milliliters of blood were collected under aseptic technique into plain container, the sera obtained after centrifugation were kept at -20 until IgG antibodies were processing by ELISA. All reagents were brought to room temperature before assaying.

Washing buffer was prepared 1:30 from buffer concentrate with distilled water. 100µl of sample diluents was added into appropriate wells except the blank well and negative well, and standard incubated the sample for 60 min at 37°C in moist chamber aspirate or shake out the incubated solution washed all wells with washing solution by automated washer (4x300 µ IDIL WASH) dry by tapping the micro titer plate on paper towel (Washing 1)

100µl of APC-Conjugate Reagent was added in to each well except the blank, the plate was mixed well and incubated for 30 min at 37°C in moist chamber. The liquid was aspirated

and each well was rinsed in wash buffer (Washing²). Solution by automated washer (4x300 μ IDIL WASH) dry by tapping the micro titer plate on paper towel 100 μ l of substrate pNPP solution was added into each well including the Blank and mixed by tapping the plate gently. The plate was incubated at 37°C for 30 min. In moist chamber 100 μ l Stop solution was added into each well and mixed gently.

Measuring the absorbance: The plate reader was calibrated read optical density (OD) at 405 nm against substrate blank. To fix the cut-off ranges multiply the mean value of the measured standard OD with numerical data of the quality control certificate. The results were calculated by relating each sample optical density (OD) value to the Cut off value of plate. The absorbance was read with micro well reader at 405nm.

Interpretation of Results:

Negative results: samples giving absorbance less than Cut-off value are negative for this assay.

Positive result: sample giving absorbance equal to or greater than Cut-off considered initially reactive.

Borderline: sample with absorbance to Cut-off value are considered borderline and retesting of these samples induplicate is recommended.

Data analysis:

The generated data were analyzed by using master sheet and Statistical Package for Social Sciences (SPSS) program. The seropositivity of CVB(IgG), and related to gender, age, and type of heart disease, were demonstrated by chi-square test and statistical significant relationship was obtained by p-value($p \leq 0.05$).

Results:

A number of 90 participants (45 patients of cardiac disease and 45 from healthy individuals control group), who attending two center of cardiac disease in Khartoum state, Sudan, were enrolled in this study, their age range 14-80 years old with mean 47 years old, out the total 45 patients with heart disease among them 29(64.4%) males, and 16(35.6%) females the result showed 31(68.9%), out of the total were positive for CBV IgG patients, out of them 21(67.7%) males and10(32.3%) were females (figure1,2), the highest seroprevalance was observed among 35-55 years age group 15(48.4%) tabel(1) , most studied population had dilated cardiomyopathy 13(28.9%) and highest CVB seropositivity was observed among them 11(35.5%) table (2), and among the duration of cardiac disease in less than month is the highest also showed 14(45.2%) tabel(3) .

Regarding 45 healthy individuals (control group) 35 (77.8%) males and10(22.2%) females out of them 19(38%) were positive for CVB IgG ,the highest seropositivity showed among males 17(89%) (figur 3) .

This study shown statistically significant relationship between CBV seropositivity among patients and controls group ($p=0.01$) table (4). However, there was insignificant relationship between the seroprevalance and the factors, age, gender, type of heart disease and duration of heart disease.

Discussion:

Several researches have been made and reported different results in various countries associated with CBV among heart disease, the study result revealed that 31(68.9%) patients were positive for CBV IgG, but no published data was available about prevalence of CBV among heart disease patients in Sudan. When compared with other findings it found to be similar to other study by Gs sainani, et al(1975) of 55 patients

with heart disease who found positive for 19 patients⁷, when has been compared with other studies the present study is low to study by Lau Rc in Newzealand, in which they found seroprevalance of CVB is 78 cardiac patients positive for CVB, and series report of 42 patients in Western Australia serological tests implicated coxsackie B viruses (B2 in 12 cases, B5 in 10 cases, B4 in 9 cases, B1 in 4 cases, B3 in 3 cases)⁸. The variation may be due to sample size, technique use for analysis, and lower than reviews by Lerner & Wilson(1973) and Abelmann (1973) (9,10)

When the present result has been compared with other study in Sudan on Diabetes mellitus, it was slightly lower to study was conducted by Emad, Ali, Enan, (2005-2007) of coxsackie virus on Diabetes Mellitus. The test result for IgG have shown 45% positives⁽¹¹⁾, that mean the coxsackie virus found in Sudan in high frequency. In the present study the relationship between seropositivty of 2 group cardiac patients and non cardiac patients the 31(62%) positive of patients and 19(38%) for non cardiac and it is significant ($p=0.011$). The seropositivty of gender is high in males 21(67.7%) Most studies report a slight male's predominance. Regarding age groups the seropositive result was high among 35-55 age range. Statistically there were no relationship between type of heart disease (infective endocarditis, STME ST segment elevation myocardial infarction, dialatedcardiomyopathy, heart failure, myocarditis, pericarditis) there was insignificant. Also, statistically, there was insignificant relationship between seropositivty and duration of cardiac disease. In conclusion this study reported high seroprevalance of CVB among heart disease patients that indicate the importance for investigation for all patients who had cardiac disease. We recommended confirmation and mentoring with large scale specimens.

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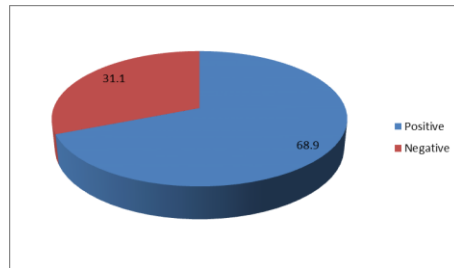


Figure 1: Seroprevalence of coxsackie B virus among cardiac patients group: (n:45)

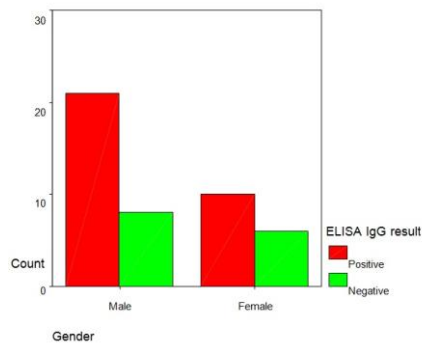


Figure 2: Seroprevalance of coxsackie B virus among patient according to their gender: (n:45)

Table 1: Seroprevalence of coxsackie B virus among patients group (n:45) according to their age

Age range in years	Positive NO (%)	Negative NO (%)	Total NO (%)	Pvalue
				.111
Less than 15	0(0%)	1(7.1)%	1(2.2%)	
15-35	3(9.7%)	5(35.7)	8(17.8)	
35-55	15(48.4)	4(28.6)	19(42.2)	
55-75	12(38.7)	4(28.6)	16(53.6)	
75-95	1(3.2)	0(0)	1(2.2)	
Total	31(68.9)	14(31.1)	45(100)	

Table 2: Seroprevalence of coxsackie B virus among heart disease patients (n:45) type of heart disease

Type of Heart disease	Positive NO (%)	Negative NO (%)	Total	P value
				.338
Infective Endocarditis	4(12.9)	0(0)	4(8.6)	
STsegment Elevation Myocardial infarction	4(12.9)	2(14.3)	6(13.3)	
Dialatedcardiomyopathy	11(35.5)	2(14.3)	13(28.9)	
Heart failure	6(19.4)	4(28.6)	10(22.2)	
Myocarditis	5(16.1)	5(35.7)	10(22.2)	
Pericarditis	1(3.2)	1(7.1)	2(4.4)	

Table 3: Seroprevalence of coxsackie B virus according to duration of cardiac disease among patients group (n:45)

Duration	Postive N.O%	Negative NO,%	Total	Pvalue
Less than 1 month	14(45.2)	8(57.1)	22(48.9)	.792
1month- less than year	10(32.3)	4(28.6)	14(31.1)	
1year-5year	4(12.9)	2(14.3)	6(13.3)	
5year-10 year	1(3.2)	0(0)	1(2.2)	
10 year and above	2(6.5)	0(0)	2(4.4)	
Total	31(68.9)	14(31.1)	45(100)	

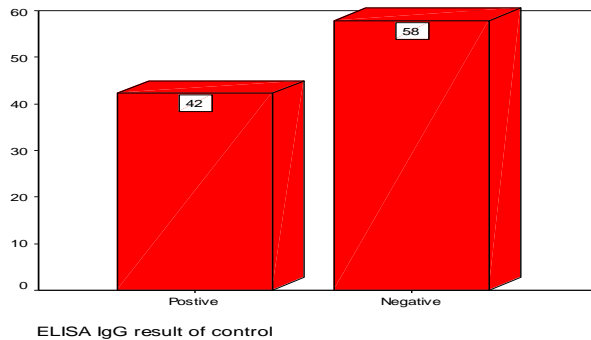


Figure 3: Seroprevalence of CVB among control group (n:45)

Table 4: Correlation between seropositive of coxsackie B virus among Patient and control group:

	Healthy individuals	Heart patient	Total	p-value
coxsackie B virus positive	19(38.0)	31(68.9)	50(55.6)	.011
coxsackie B virus negative	26(65.0)	14(35.0)	40(44.4)	
Total	45(100)	45(100)	90(100)	

*P < 0.05 is significant.

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