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SHORT COMMUNICATION

# Seroprevalence of hepatitis C virus antibodies among Sudanese patients with schistosomiasis referred to Al-elafon military hospital in Khartoum state

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## Abstract

**Background:** Hepatitis C virus (HCV) infection and schistosomiasis, has worldwide coexistence especially in Africa. Some researchers suggest that schistosomiasis is the risk factor for the development of HCV infection.

**Objective:** The current study was aimed to determine seroprevalence of HCV among Sudanese patients with schistosomiasis.

**Method:** From April 2017 to July 2017, a total of 60 blood samples were obtained from patients who confirmed microscopically with schistosomiasis. The blood samples were centrifuged at 3000 RPM for 5 minutes to obtain serum. All serum samples were screened for the presence of HCV IgG antibody by using indirect ELISA. The samples with positive reaction were confirmed by repeating the test. We used an interviewer-administered questionnaire to ask participants about their demographic data as well as their geographical affliation. Statistical analysis was performed by using SPSS version 20.

**Result:** All patients were male and aged between 15 to 27 years old with an average of 20.1± 2.25 years. Out of 60 serum samples investigated 3[5%] were positive for HCV IgG antibody, while 56[93.3%] were shown a negative result. Interestingly, we determine one sample 1[1.7%] with borderline reaction.

**Conclusion:** The study concluded that there was a high seroprevalence of HCV IgG antibody among patients with Schistosoma infection in comparison to the finding of previous researchers who investigate those are not infected. This may suggest a possible association between HCV infection and Schistosoma. Further studies with the inclusion of large sample size and by using a more advanced technique [PCR] should be considered in the future.

***Keyword:*** HCV; schistosomiasis; ELISA

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## Introduction

Hepatitis C virus (HCV) was initially isolated from the serum of a person with non-A, non-B hepatitis in 1989 [1]. The HCV is an RNA virus that belongs to the family *Flaviviridae* [2]. The structure of HCV consists of a lipid membrane envelope that is 55 to 65 nm in diameter. The viral envelope consists of two glycoproteins types, E1 and E2, which are embedded in the lipid envelope [3, 4]. The envelope is enclosed an icosahedral core that is 33 to 40 nm in diameter inside the core is the RNA material of the virus [3]. The genome consists of a single open reading frame that is 9600 nucleotide bases long. Based on genetic differences between HCV isolates, the hepatitis C virus species is classified into six genotypes [1–6] with several subtypes within each genotype (represented by lower-case letters). Subtypes are further broken down into quasispecies based on their genetic diversity. Genotypes differ by 30–35% of the nucleotide sites over the complete genome [5, 6]. It is estimated that 143 million people [2%] of people globally are living with chronic hepatitis C [6]. About 3–4 million people are infected per year, and more than 350,000 people die yearly from hepatitis C-related diseases [7]. During 2010 it is estimated that 16,000 people died from acute infections while 196,000 deaths occurred from liver cancer secondary to the infection [8].

The rates of infections are high (>3.5% population infected) in central and East Asia, North Africa and the Middle East, they are intermediate (1.5%-3.5%) in South and Southeast Asia, sub-Saharan Africa, Andean, Central and Southern Latin America, Caribbean, Oceania, Australasia and Central, Eastern and Western Europe; and they are low (<1.5%) in Asia-Pacific, Tropical Latin America and North America. The total number of people with this infection is higher in some countries in Africa and Asia. Countries with particularly high rates of infection include Egypt [22%], Pakistan [4.8%] and China [3.2%]. It is believed that the high prevalence in Egypt is linked to a now-discontinued mass-treatment campaign for schistosomiasis, using improperly sterilized glass syringes [9].

Schistosomiasis, also known as snail fever and bilharzia, is a disease caused by parasitic flatworms called schistosomes. The urinary tract or the intestines may be infected. Symptoms include abdominal pain, diarrhea, bloody stool, or blood in the urine. Those who have been infected for a long time may experience liver damage, kidney failure, infertility, or bladder cancer. In children, it may cause poor growth and learning difficulty [10].

The genus *Schistosoma* Over twenty species are recognised within this genus. The genus has been divided into four groups: *indicum, japonicum, haematobium and mansoni*. The affinities of the remaining species are still being clarified. *S.haematobium* usually infect the urinary system, while *S. mansoni* inhibits intestinal tract [11].

Schistosomiasis is a parasitic infection that is second to malaria in prevalence and affects about 200 million people in over 70 countries with an infection rate of one in 30 individuals. Also, it is of particular important in Africa and South America owing to it is endemicity, high prevalence and morbidity rates in countries such as Nigeria, Tanzania, Democratic Republic of Congo, Ghana, and Brazil [12-14].

In 2010, approximately 238 million people were infected with schistosomiasis, 85 percent of whom live in Africa. An estimated 600 to 700 million people worldwide are at risk from the disease because they live in countries where the organism is common. In 2012, 249 million people were in need of treatment to prevent the disease. Estimates regarding the number of deaths due to schistosomiasis are varied. Worldwide, the Global Burden of Disease Study issued in 2010 estimated 12,000 direct deaths. While the WHO in 2014 estimated more than 200,000 annual deaths related to schistosomiasis [15, 16].

There are many contradictory data about the prevalence of HCV/ *Sichtosoma* co-infection in endemic areas and the risk factors associated with increased susceptibility for HCV infection in a patient with schistosomiasis. Some researchers suggest that schistosomiasis is the responsible factor, either by producing false positivity for HCV antibodies or by predisposing to actual HCV infection in some way [17].

In Sudan, most previous studies were focused on the prevalence of HCV in the general population as epidemiological researches. Since there are no more data available about the association between the virus and schistosomiasis the current study was aimed to determine seroprevalence of HCV

among Sudanese patients with schistosomiasis. By comparing the result obtained from this study with the data obtained from non-Schistosoma infected patient we can determine also the correlation existences.

## Methods

This study was cross-sectional. It was conducted in Alelafon military hospital in Khartoum State. The practical part of this study was done in the Research Laboratory, Sudan International University, during the period of April 2017 to July 2017. A total of sixty blood samples (n=60) were obtained from patients with schistosomiasis confirmed microscopically, all patients were males and no females were included.

### *Specimens' collection and processing*

A total of 60 urine and stool samples were collected from patients to confirm Schistosoma infection. After infection confirmation, a respective volume of 5 ml blood was collected from each patient through then displaced into a plain container. Each blood sample was centrifuged at 3000 g for 5 min., and then serum was gently collected into Eppendorf tube and stored at –20°C until the serological analysis.

### *Microscopical examination of stool and urine samples for Schistosoma eggs*

A single stool and urine samples were collected from all consented study participants using labeled clean containers. Wet preparation was used for both urine and stool samples according to Cheesbrough parasitological tests [18]. Schistosoma eggs were determined by 10X magnification then the result was recorded.

### *Anti- HCV antibody detection by ELISA Test*

All of the anti-HCV antibody by a commercially available enzyme-linked immune-sorbent assay "anti-HCV ELISA" kit (Fortress Diagnostics Limited, unit 2C Antrim Technology Park, Antrim, BT4I IQS United Kingdom) [19]. The assays were performed following the instructions of the manufacturer. Positive and negative controls were included in each assay. According to the information included in the kit's insert, the immunoassay used has a specificity of 99.94% [19]. Each positive result was confirmed by retesting the sample.

### *The procedure of the ELISA test*

All reagents and specimens were settled to reach room temperature and the ELISA procedure was done following manufacture instruction (Fortress Diagnostics Limited, unit 2C Antrim Technology Park, Antrim, BT4I IQS United Kingdom).

### *Quality control and calculation of the results*

Reagent, standard, and control were checked for storage, stability, and preparation before starting work. Each microplate was considered separately when the results were calculated and interrelated; the results were calculated by relating each specimen absorbance [A] to the cut off [c.o.) of the plate. Cut off value was calculated through the equation of [C.O.) = *NC + 0.12 (*NC is mean of the three negative controls). The OD value of the blank was less than 0.080 at 450 nm. The OD value of the positive control was more than 0.80 at 450 nm. The OD value of the negative control was less than 0.1 at 450 nm.

### *Interpretation of results*

Positive more than cut-off value. Negative less than the cut-off value. Borderline: samples with absorbance O.D. <= Cut-off *2 are considered borderline and retesting of those samples in duplicates is recommended.

### *A method used for data collection*

Data were collected by using administrated questionnaire including the gender and age.

### *Data analysis*

The data collected from the questionnaire and laboratory results were analyzed by SPSS version 20 computerized programs.

## Results

In this study 60 serum sample was collected from patients with Schistosoma infection take from Al-elafon military hospital. All patients were out patient and male without any symptoms and sign of hepatitis C virus infection. The patient stays in AL-elafon camp for at least three months; our study group was aged between 15-27 years old with a mean of 20.1833±2.25 years (Table 1). The patients were referred to the hospital from different area including Khartoum state 41 patients (68.3%), Blue Nile state15 patients (25%) and 2(3.3%) patients were referred from Aljazeera as well as 1(1.7%) patient from Shandi and another one from South Kordofan (Table 2).

**Table 1:** Describe the patients age.

| | | |
|---|---|---|
| *N* | *Valid* | *60* |
| | *Missing* | *0* |
| Mean | | 20.1833 |
| Median | | 20.0000 |
| Std. deviation | | 2.25863 |
| Minimum | | 15.00 |
| Maximum | | 27.00 |

**Table 2:** Describe patients' geographical affiliations.

| | | *Frequency* | *Percent* | *Valid percent* | *Cumulative percent* |
|---|---|---|---|---|---|
| Valid | Aldamazeen | 15 | 25.0 | 25.0 | 25.0 |
| | Aljazeera | 2 | 3.3 | 3.3 | 28.3 |
| | Bahry | 3 | 5.0 | 5.0 | 33.3 |
| | Khartoum | 5 | 8.3 | 8.3 | 41.7 |
| | Omdurman | 33 | 55.0 | 55.0 | 96.7 |
| | Shandi | 1 | 1.7 | 1.7 | 98.3 |
| | Southkurdfan | 1 | 1.7 | 1.7 | 100.0 |
| | Total | 60 | 100.0 | 100.0 | |

The result shows that out of 60 serum samples investigated 3(5%) were positive for HCV IgG antibody, while 56(93.3%) were shown a negative result. Interestingly, we determine one sample 1(1.7%) with borderline reaction (Table 3).

**Table 3:** Result of Anti *HCV* ELISA test.

| | | *Frequency* | *Percent* | *Valid percent* | *Cumulative percent* |
|---|---|---|---|---|---|
| Valid | Negative | 56 | 93.3 | 93.3 | 93.3 |
| | Positive | 3 | 5.0 | 5.0 | 98.3 |
| | Borderline | 1 | 1.7 | 1.7 | 100.0 |
| | Total | 60 | 100.0 | 100.0 | |

## Discussion

Over decade there are many contradictory data about the prevalence of HCV among Schistosoma patients in endemic areas and the risk factors associated with increased susceptibility for HCV infection in patient with schistosomiasis the controversial finding about the impact of schistosomiasis as risk factor for HCV infection could be indicated by the statement that the prevalence rates of HCV antibody with wide variations as low as 1% and as high as 50% among patients with schistosomiasis, were reported in different countries [17]. Since the HCV/ *Sichtosoma* co infection very reaches researchable material, the impact of the recent study was to highlight the need for extensive researches in this field in Sudan.

In our study, we find that the prevalence of HCV antibody among *Schistosoma* patients was 5% which is higher than that among normal non schistosomal patients 0-45% [20]. And also higher than that obtained by Hatim et al., in 2007 in Aljazeera state who reported that the prevalence of HCV antibody was 2.2% [21]. The prevalence seemed to be increased over 10 years from 2007. Instead, the prevalence was found to be lower than that noted in patients with end-stage renal disease on regular hemodialysis with a seroprevalence of 7% [22].

The high percentage of HCV infection rates among patients with schistosomiasis in comparison to those who non infected and to the prevalence for those in the previous studies may suggest the possible association between HCV infection and Schistosoma and the schistosomiasis is coming directly after renal hemodialysis as most factors that bearing a high risk for developing HCV infection. However, we use a serological technique which may relatively appear to be less sensitive than PCR. So, we recommend the later mentioned technique in further studies to get a more reliable result.

Interestingly, since the fact that Egypt is closely related to Sudan geographically we must compare our finding with their reports. The seroprevalence of anti HCV among Egyptian Schistosoma patients was 67% which is markedly higher than our prevalence in our study. These discrepancies in the prevalence although the closeness between our country and Egypt may need more attention and investigations from researchers interested in this field.

## Conclusion

The study concluded that there was a high seroprevalence of HCV IgG antibody among patients with Schistosoma infection in comparison to the finding of previous researchers who investigate those are not infected. This may suggest a possible association between HCV infection and Schistosoma. Further study with the inclusion of large sample size and by using a more advanced technique (PCR) should be considered in the future.


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## Author contributions

All authors contributed equally to this work. In concept, design, data collection and processing and writing the final document. All co-authors have seen and approved the final version of the paper and have agreed to its submission for publication.

## Conflict of interest

The authors declare that they have no conflict of interest

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