Applied Medical Research, 2019
VOL 6, NO. 2, PAGE 1 – 6
DOI: doi.org/10.47363/AMR/2019(6)167

ORIGINAL RESEARCH

Open Access

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 have worldwide coexistence, especially in Africa. Some researchers suggest that the 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 to July 2017, 60 blood samples were obtained from the patients who confirmed microscopically with schistosomiasis. The blood samples were centrifuged at 3,000 RPM for 5 minutes to obtain serum. All serum samples were screened for the presence of HCV IgG antibody by using Indirect Enzyme Linked Immunosorbent assay (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 afflation. Statistical analysis was performed by using Statistical Package for the Social Sciences (SPSS) version 20.

Result: All patients were male and aged between 15 and 27 years old with an average of 20.1 ± 2.25 years. Out of 60 serum samples investigated, three (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 a large sample size and by using a more advanced technique Polymerase Chain Reaction (PCR) should be considered in the future.

ARTICLE HISTORY

Received July 24, 2019
Accepted November 19, 2019
Published November 27, 2019

KEYWORDS

HCV, schistosomiasis, ELISA

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. The HCV was first identified in 1989 and was found to be the cause of 80%–90% of cases of non-A, non-B hepatitis. HCV is classified in the *Hepacivirus* genus within the Flaviviridae family [2].

The structure of HCV consists of a lipid membrane envelope that is 55–65 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 33–40 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 9,600 nucleotide bases long. This single open reading frame is

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translated to produce a single protein product, which is then further processed to produce smaller active proteins. Based on genetic differences between HCV isolates, the HCV species are 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. The difference in genomic composition of subtypes of a genotype is usually 20%–25%. Subtypes 1a and 1b are found worldwide and cause 60% of all cases [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]. Among those chronically infected, the risk of cirrhosis after 20 years varies between studies but has been estimated at ~10%–15% for men and ~1%–5% for women. The reason for this difference is not known. Once cirrhosis is established, the rate of developing hepatocellular carcinoma is ~1%–4% per year [7].

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 [9]. 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 [10].

The genus *Schistosoma* over 20 species are recognized 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. *Schistosoma haematobium* usually infects the urinary system, while *S. mansoni* inhibits intestinal tract [11,12].

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 1 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 [13–17].

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 [18].

The data published about HCV and schistosomiasis co-infection is few. In a systematic review done by Gasim et al. [19], they include only 16 articles worldwide which fulfill their study criteria. Prevalence rates of HCV infection with wide variations as low as 1% and as high as 50% among patients with schistosomiasis were reported in different countries [19]. A more recent study in Egypt among 3,596 patients found that 27.3% had both HCV-RNA and schistosomiasis [20]. Nonetheless, in Yemen a group of researchers reported a relationship between *S. haematobium* and hepatitis B, but no correlation between *S. mansoni* infection and HBV or HCV [21]. The prevalence of *S. mansoni* infection was 65.9% in a community-based study in Ethiopia, including 2,451 subjects [22].

In Sudan, most previous studies were focused on the prevalence of HCV in the general population as epidemiological research studies. 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.

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, from the period of April to July 2017.

Specimens' collection and processing

Sixty 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 30,00 g for 5 minutes, 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 sample urine and stool 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 [23]. 10× magnification determined *Schistosoma* eggs then the result was recorded.

Anti- HCV antibody detection by ELISA test

All patients samples were examined for the presence of 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) [24]. 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% [24]. 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, 100 µl of specimen diluents was added to each well except the blank then 10 µl of a positive control; negative control and specimen were added to their respective wells. The plate was covered with plate cover and incubated for 30 minutes at 37°C. At the end of the incubation period, each well was washed five times with diluted wash buffer. Then, 100 µl of Horseradish peroxidase (HRP) conjugate was added to each well except the blank well. Then, the plate was covered with plate cover and incubated for 30 minutes at 37°C. Again, at the end of the incubation period, each well was washed five times with diluted wash buffer. Finally, 50 µl of chromogen A and chromogen B solutions were added to each well including blank, then the plate was incubated at 37°C for 15 minutes and stop solution was added.

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).

Quality control reading in ELISA test

The odd-Ratio (OD) value of the blank was less than 0.080 at 450 nm. The OD value of the positive control was 0.80 at 450 nm. The OD value of the negative control was 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 SPSS version 20 computerized programs analyzed laboratory results.

Results

Sixty blood samples (n = 60) were obtained from patients with *Schistosomiasis* confirmed microscopically. All patients were male with no symptoms and sign of HCV infection. The patients stay in AL-elafon camp for at least 3 months; our study group was aged between 15 and 27 years old with an average of 20.18 ± 2.25 years (Table 1). Patients were referred to AL-elafon hospital from a different regions of Sudan including Khartoum state 41 patients (68.3%), Blue Nile state15 patients (25%) and 2 (3.3%) patients were transferred from the Aljazerra state as well as 1 (1.7%) patient from

Table 1. Describe the patients age.

N	**Valid**	**60**
	Missing	**0**
Mean		20.1833
Median		20.0000
Stdandard 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	

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	

Chandi provenance and another one from South Kordofan state (Table 2).

The result shows that out of 60 serum samples examined only three [5%] were positive for HCV antibodies, while 56 [93.3%] showed a negative results. It is interesting to determine one sample 1 [1.7%] with the borderline result (Table 3).

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. The data published about HCV and *Schistosomiasis* co infection is few. In a systematic review done by Gasim et al. [19], they include only 16 articles worldwide which fulfill their study criteria. Prevalence rates of HCV infection with wide variations as low as 1% and as high as 50% among patients with Schistosomiasis were reported in different countries [19]. A more recent study in Egypt among 3,596 patients found that 27.3% had both HCV-RNA and Schistosomiasis [20]. Nonetheless, in Yemen a group of researchers reported a relationship between *S. haematobium* and hepatitis B, but no correlation between *S. mansoni* infection and HBV or HCV [21]. The prevalence of *S. mansoni* infection was 65.9% in a community-based study in Ethiopia including 2,451 subjects [22]. 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 compare our results with the data obtained from non-Schistosoma infected patient to determine the correlation existences. We find that the prevalence of HCV antibody among Schistosoma patients was 5% which is higher than that among normal non-Schistosomal patients 0%–4.0 % the later percentage was reported from meta-analysis study that conducted about the Sudanese HCV antibody prevalence during the period from 1996 to 2008 including about nine studies and 1,800 participants from different categories of population, namely, general population, blood donor, and pregnant women all the patients were examined by using Enzyme Linked Immunosorbent assay (ELISA) kit [25]. In addition, our result was higher than that obtained by Hatim et al. [26] in Aljazeera state who reported that the prevalence of HCV antibody was 2.2%. The prevalence seemed to be increased over 10 years from 2007. Instead, the prevalence found to be lower than that noted in patients with end-stage renal disease on regular hemodialysis with a seroprevalence of 7% [27].

The relatively high HCV infection rates among patients with Schistosomiasis compared to those normal population infection rates and to the prevalence for those in the previous studies, which were, used the same technique that we used may suggest the possible association between *HCV* infection

and *Schistosoma*. However, we use a serological technique, which may relatively appear to be less sensitive than Polymerase Chain Reaction (PCR). Therefore, 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 [19]. 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.

It is worth to mention that in our study, we determine one sample 1 [1.7%] with borderline reaction meaning that the patient is not positive but he is still suspicious case because the antibody is present in the serum of patient but it did not reach appositive detectable level by using ELISA test which may need a retesting duplicates after 2–4 weeks so we can get a clear cut result.

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 future studies. In addition, confounding factors, which may affect the correlation between the *HCV* and *Schistsomasis*, could also be considered in future studies.

Acknowledgments

The author thanks extend to all those help us to create this research. A lot thanks and appreciates to all the patients who participated in this study, with our best wishes for them to be well and good as soon as possible.

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.

Funding

There was no funding received for this study.

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