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TRANSFUSION RELATED HEPATITIS C VIRUS ANTIBODIES AND POSSIBLE RISK FACTORS IN HEALTHY BLOOD DONORS

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ABSTRACT

The main cause of transfusion associated hepatitis, cirrhosis and hepatocellular carcinoma is HCV infection. The present research was carried out to investigate the outbreak extent of anti–HCV antibodies among healthy blood donors attending the transfusion department at Khartoum hospitals. 3000 volunteer healthy blood donors participated in a cross–sectional study. Rapid test method was applied and positive samples were approved with the ELISA (fourth generation). SPSS software version 26 was used for statistical analysis of data. 0.5% of the research population was detected to have anti-HCV antibody. Donors (all male) were between 19–46 years old with mean age (1.95± 0.421SD). 2545 (84.8%) were volunteer, 455 (15.1%) were family replacement donors, 11 (0.36%) were positive among volunteer group, and most of the participants (0.16%) who were diagnosed with hepatitis C antibodies had history of cupping. 2265 (75%) of donors had a history of jaundices; only 13 (0.6%) statistically carried antibodies against HCV (P value 0.012). Sudanese blood donors had a low seroprevalence of hepatitis C virus; however, the existence of the virus and the likelihood of transmission and dissemination were confirmed. Hence, very rigorous guidelines are necessary for selecting blood donors. Furthermore, laboratory diagnostic tests for identifying infectious agents must be significantly enhanced.

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Introduction

Around 170 million individuals are diagnosed with the hepatitis C virus (HCV), with about 3.5 million new cases occurring per year, causing viral HCV along with HBV infection is among the world's most serious blood born infectious disease concerns [1]. Because of the hazard of transfusion-transmitted infection, whole blood is evaluated before transfusion for five markers: Syphilis, malaria, HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV) [2]. Hepatitis C virus [HCV] is the main causative agent of post-transfusion hepatitis [3-6], and the World Health Organization (WHO) reports that 3% of the world's population is severely infected with HCV, with the majority of these cases occurring in Africa, which has the highest HCV seroprevalence [7]. Hepatitis C virus is spread globally with prevalence differing from 0.2% up to 40% in various countries; approximately, 21.3 million of HCV carriers are in the Eastern Mediterranean countries [8, 9]. Almost known routes of transmission include intravenous injection, blood transfusion, sexual intercourses, and exposures to contaminated medical practices [10]. Controlling and prevention of HCV infection, explaining its geographic distribution, diagnosing its risk factors, and investigating co-factors which accelerate infection progression are almost complicated. The crude seroprevalence of significant blood borne infectious agents between voluntary blood donation was 17.6 percent**,** and

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post-transfusion hepatitis affecting nearly 12.5 percent of patients who are receiving a blood transfusion [11]. HCV and other transfusion-associated infections have been significantly decreased in the countries where routine serologic screening of blood donation has been implemented, such as in North European and South European where prevalence of HCV was (0.01% - 0.02%), and (1-1.5%), respectively [12]. Because of problems such as unsafe medical practices, low quality in blood screening tests, and intravenous drug abuse with shared needle, HCV is more prevalent in developing countries. For example, higher HCV prevalence rate has been reported in Southeast Asian countries, including India [1.5%], Malaysia (2.3%), Philippines (2.3%), Pakistan (8.1%), and in equatorial Africa (6.5%), and as high as (20%) in Egypt [11, 12].

Since the developed countries implement more sensitive tests that detect infection earlier, they reduce risks of transfusion transmitted viral infection [13]. Demand for increasing blood transfusion control is crucial specifically in developing countries that is known with highest endemicity of blood borne infections like HIV, HBV, HCV, malaria, to diminish nutritional problem and obstetrical malaria, nutritional problem and obstetrical blood loss [14-16]. In Sudan viral hepatitis antibodies were 3% of the patients investigated in comparison to 82% positive for Hepatitis B virus [HBV] markers [17]. The present research aimed to determine the prevalence of hepatitis C virus antibodies through screening of healthy blood donors in Khartoum state – Sudan.

Materials and Methods

Study Design

This is a descriptive cross-sectional study for detection of anti-hepatitis C virus antibodies in healthy blood donors in Khartoum state – Sudan. Ethical clearance was given by Al-Fajr College for Science and Technology, the purpose of the study was explained to each of the participants, as well as the director of lab of hospitals, and the informed consent of donor were taken implicit according to the blood donation protocol.

Study Area and Population

The research was done in the blood bank of three hospitals in Khartoum state, Military hospital helipad and General Omar Sawi hospital and Bahari hospital, during 7 months (from August 2020 to February/2021); 3000 donors participated in the study, all of whom were over 18 years old, and were interviewed by trained data collector team; a questionnaire was used to collect data on age, living place, and family relationship with patients (in case of family replacement donors), job, and blood donation history.

Methods

The methods used for usual screening of voluntary blood donors in the blood banks of the hospitals in Sudan are: the rapid screening test, fourth generation of ELISA, and Polymerase chain reaction (PCR). In the present research the one step cassette style Anti-HCV serum/plasma Test, ABON HCV (Catalog number-IHC-302) was applied. The sealed pouch was opened, the cassette test kit was removed from the pouch and used as soon possible. Using a pipette, we added 50 microliter of serum to the specimen well of the test device and then added 1 full drop of buffer (30 microliter) and started the timer. The result was read after 10 minutes of incubationin in room temperature.

The positive result was approved by HCV Ab fourth generation Enzyme Linked immunosorbent assay (ELISA). The stock washbuffer was diluted 1 to 20 with distilled water. We set the strip that was needed in strip-holder, included three negative control and two positive control and one blank, in blank well we added sample or HRP-Conjugate, then, added 100 microliter of specimen diluent into each well except the blank; after that, we added 10 microliter of positive control and negative control and specimen into their respective wells, and used separate disposal pipette tips to prevent cross-contamination, and after that covered the plate with plate cover and incubated for 30 minutes at 370 C using dry incubator, after the end of incubation, we removed the plate cover and washed the well 5 times by diluted wash buffer each time, allowed the micro wells to soak for 45 seconds; after the end of cleaning, turned the strip onto blotting paper to remove any remainders. Then, we added 100 microliter of HRP-Conjugate to each well expect the blank, covered it and incubated for 30 minutes for 37 °C. Bythe end of incubation, we remove the plate cover and washed it 5 times with diluted wash buffer. In coloring stage we dispensed 50 microliter of chromogen A and 50 microliter chromogen B into each well including the blank and mixed gently. Then, we incubated the plate at 37 °C for 15 minutes and avoid light (the enzymatic reaction between the chromogen A/B solution produce blue color in positive control and anti-HCV positive sample well). Using multichannel pipette we added 50 microliter of stop solution into each well and mixed gently, after 5 minutes of stop reaction we calibrated the plate reader with the blank well and read the absorbance at 450 nm; the results are calculated by relating each samples optical density value to the cut-off value of the plat. (Fortress diagnostics Kits) are used.

Statistical Analysis

The data wereentered in a master sheet and analyzed by the Statistical Packages for Social Sciences (SPSS) version 26. Chi square was used where the statistical significance level was set at p 0.05, and descriptive data was presented in numbers and percentages.

Results and Discussion

The total donors was 3000 Sudanese males, HCV antibodies were detected in 15 (0.5 %) of subjects participated **(Table 1)**. Ages of donors ranged from 19-46 years old with the mean age (1.95±0.421SD); participants were distributed in four age groups, majority of donors 1295 (43.16 %) were in age group between 26-32 years old**,** where 6 (0.2%) of them were positive for HCV antibodies. 56.7% of the donors resided in Khartoum state; among them 0.3% were positive for HCV antibodies. Obviously (0.4%) of HCV seropositive of donors have had a history of jaundice. On the other hand, 2545 (84.8%) was volunteer, 455 (15.1%) was family replacement donors, and 11 (0.36%) were positive among volunteer group. 103 (3%) was illiterate, 514 (17.1%) had secondary, and 2383 (79%) had university education. Fortunately, (51.9%) of subjects had no history of blood transfusion or any related risk factors, while most of the participants (0.16%) who were diagnosed with hepatitis C antibodies had history of cupping, all data were summarized in **Table 2**. **Table 3** displayed the correlation between different risk factors and history of jaundice with HCV antibodies; a statistically significant difference was revealed between donors with history of jaundice and seropositivity of HCV as (P value 0.012); as among 2265 (75%) of donors had history of jaundices only 13 (0.6%) carried antibodies against HCV. No significant result was detected between risk factors and HCV (P value 0.315).

Table 1. Prevalence of Hepatitis C Virus among Blood Donors

	Frequency of Blood Donors	**Percentage (%)**
Positive	15	0.5%
Negative	2985	99.5%

Table 2. Baseline Data of Blood Donors

Parameters	**Total blood Donors n=3000**	**Percentage of Blood donors (%)**	**Frequency HCV positive**	**Percentage of HCV Positive (%)**
Age group				
19 - 25 years old	85	28.4 %	4	0.13%
26 – 32 years old	1295	43.16 %	6	0.2%
33 – 39 years old	579	19.3 %	4	0.13%
40 – 46 years old	254	8.64 %	1	0.03%
Residence				
Khartoum state	1703	56.7%	9	0.3%
Other states	1297	43.2%	6	0.2%
Blood donation status				
Anonymous volunteers	2545	84.8%	11	0.36%
Family replacement donors	455	15.1%	5	0.16%
Educational level				
Illiterate	103	3%	13	0.44%
Secondary	514	17.1%	1	0.03%
University	2383	79%	1	0.03%
History of Jaundice				
Yes	2265	75.5%	12	0.4%
No	735	24.5%	3	0.1%
Risk factors				
No	1558	51.9%	3	0.1%
Blood transfusion	156	5.2%	2	0.06%
Wet Cubing	708	23.6%	5	0.16%
Others	578	19.2%	4	0.13%
Total	**3000**	**100%**	**15**	**0.5%**

Table 3. Correlation Between Different Risk Factors and History of Jaundice with HCV Antibodies

parameters	Risk factors n=1442 (%)			P. value	History of Jaundice n=3.000 (%)		P. value
	Wet cupping n=708 (%)	Others n=578 (%)	Blood transfusion n=156 (%)		Yes n=2265 (%)	No n=735 (%)	
Positive HCV	5 (0.7%)	4 (0.7%)	2 (0.3%)	0.315	13 (0.6%)	3 (0.4%)	0.012
Negative HCV	703 (99.3%)	574 (99.3%)	154 (98.7%)		2252 (99.4%)	732 (99.5%)	
Total	708 (100%)	578 (100%)	156 (100%)		2265(100%)	735(100%)	

The preventative measures of transfusion-transmitted infectious agents have indeed been accomplished in industrialized nations through decreasing unneeded blood transfusion, and just using regular voluntary donors, with exception of donors with specific risk factors, and monitoring all donated blood for infectious disease on a consistent basis. Inversely, in many developing countries not the slightest bit of such interventions is applied and the hazardous of transfusion-transmitted infectious pathogens are elevated, since that current study was designed to screen healthy blood donors attending different blood bank at Khartoum states for anti HCV antibodies.

The present study revealed that the outbreak rate of anti HCV antibodies among healthy Sudanese blood donors was (0.5%) which is low in comparing with previous published study in Sudan. According to Aballa TM *et al.* from Eastern Sudan in 2012 [18] and Mudawi HM from Central Sudan Geziera state in 2015 [17] the higher prevalence rates among asymptomatic Sudanese population were about 3.1% and 2.2%, respectively. In contrast to the study done by [19] at Elodeid Hospital's blood bank (2008) western Sudan, screening outcomes for anti-hepatitis C virus were negative in all blood samples tested. However, our findings are hassling to recent study in Darfur state western Sudan done by Ahmed MAI *et al.* [20] who report frequency of HCV antibodies in blood Donors was (0.4%). There is a discrepancy in the percentage of hepatitis C virus in the different states of Sudan, the highest in the state of eastern Sudan. This difference may be attributed to several reasons, including the size of the samples, the distribution of the population in the state, the type of analysis used in the study, and also the practice of some customs and habits that cause bleeding and requires preventive procedures, such as Al-Battan and wet cupping (Al-Hijama), which are widely spread in certain states in Sudan. Hence such practices may increase the prevalence of hepatitis C which may justify higher rate of BCV in previous studies and an alarming sign, it seems that an effective preventive measures have been taken to prevent the spread of blood borne transmitted infectious pathogens in the community which was evident by the decrease in the proportion of hepatitis C in the blood banks in recent studies and in our study as well.

Globally, our finding was also less than the researches carried out in Egypt (10%) [21], Nigeria (3.6%) [22], Ethiopia (1.3%) [23], and India (0.39%) [24]. These variations, and heterogeneity could be attributed to some possible racial and socioeconomic differences between the different areas of the studies, dangerous aggressive behavior, life style, and level of health-care quality and standardization or some of them were performed with the diagnosis of both hepatitis C virus RNA and anti HCV antibody.

All subjects screened in the present study were males, this is because females rarely donate blood in Sudan, that is attributed to socially and culturally concepts, and women are not favored to donate blood. Hence, those who were examined do not represent the whole population and the findings should not be generalized. In fact, donors in age group 26- 32 years old represent 43.16 %, that among them (0.2%) were infected with HCV antibodies. Our finding was consistent with to a survey done by Akbar HO [25], who concluded that the HCV infection initiates in the early age of life. The results of this study demonstrated that more than half 56.7% of blood donors were Khartoum residents rather than those who came from other states. This is because Khartoum state residents are more near and around the hospitals and can easily get access to the blood banks than other states.

The observed frequency rate of HCV infections in relation to education level was highest among illiterate people (0.44%); this may be attributed to the lack of information and knowledge about HCV virus and the route of transmission. Todd CS *et al.* [26] who suggests that anyone with lower levels of education have such a greater incidence of anti-HCV antibodies, a considerable relationship between education level and HCV was confirmed by Pereira LM *et al.* [27], so level of education of blood donors implies as potential risk factors for getting HCV; and raising the level of awareness and knowledge among the population regarding the modes of transmission of hepatitis C and complications such as liver cirrhosis, malfunction ending with liver carcinomas is contraindicated malfunction of liver and liver carcinomas. Interestingly, positive significant correlation was revealed between HCV and history of jaundice (P. value $\leq$0.012). With regard possible risk factors, unfortunately almost 0.29% of study subjects positive for anti HCV antibodies have had history of wet cupping and other procedures, these observation could justify the high proportion of HCV-infected blood donors who had low education level, as these variables are considered hazardous risk factors; however, insignificant result was demonstrated (P. value $\geq$0.315), our finding is in agreement with Sohn HS *et al.* [28].

On the other hand, the rapid diagnostic test (ICT) is a laboratory technique of choice which is an adopted protocol followed in Sudanese blood banks for screening of donated blood and then only positive results are confirmed by enzyme linked

immunosorbent assay (ELISA) which are less sensitive and low specific in compare with advance technology which depend on nucleic acid amplification tests [29]. So the prevalence rate in our study may be less than detected value when we use molecular RNA for conformation of positive HCV antibodies.
Lastly, screening of blood donors for HCV antibodies is mandatory, and infection control practices should be implemented and sustained. The opportunity of spreading HCV infection via the blood transfusion must always be regarded, and all attempts should be made to avoid it. Proper screening of blood and donor selection will be crucial to ensure a safe blood supply as no vaccine has been invented for HCV so far.

Significant Statements
The limitations of this study included the absence of some high risk factors for hepatitis C, occupation of participants, as well as sample size of study that does not represent all Sudanese population. The participants of this study were only males. We should point out that including females could have modified the results of this study. The nucleic acid amplification tests are more sensitive and able to withstand infectious agents even during the window period, which is not applicable in this study.

Conclusion

Seroprevalence of hepatitis C virus was low among Sudanese blood donors; however, the existence of the virus and the likelihood of transmission and disseminated are confirmed. Hence, very rigorous guidelines are necessary for selecting blood donors. Furthermore, laboratory diagnostic tests for identifying infectious agents must be significantly enhanced.

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