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Seroprevalence of Hepatitis C Virus Antibodies Amongst Blood Donors in Ibadan, Southwestern, Nigeria

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Abstract: This study was carried out to determine the prevalence of antibodies to HCV among blood donors in Ibadan and to generate data which may be useful to appropriate health authorities. The subjects included in this study were 200 (184 males and 16 females; aged 20 to 60 years) blood donors in the blood bank of UCH, Ibadan who consented voluntarily after thorough explanation of the purpose of the study. The samples were tested for the presence of antibodies to HCV using rapid ELISA technique developed by RIGHTCHOICE Diagnostics, Israel. Analysis of the result showed that 16 (8.0%) of the donors had antibodies to HCV. The result also showed highest HCV antibody prevalence of 11.5% amongst blood donors in the age range 30-39 years. Statistical analysis, however, showed no significant difference (p>0.05) between the prevalence rates of the male and female individuals. There was, however, no statistical association (p > 0.05) between age of the patients and prevalence of HCV antibodies. Our findings further confirm the presence of hepatitis C virus infection among blood donors in Nigeria. Routine HCV screening of blood donors is therefore recommended in order to reduce the risk of post transfusion hepatitis C.

Key words: Antibodies · Blood bank · Blood donors · HCV · Seroprevalence · Prevalence rate

INTRODUCTION

The term hepatitis C virus (HCV) was first adopted in 1989 following the identification of an RNA viral genome in a random-prime cDNA library derived from a human plasma sample containing the putative non-A, non-B hepatitis agent [1]. Epidemiological studies established that there were two routes of transmission of non-A, non-B hepatitis. Thus enteric and parenteral or post transfusion forms were recognized. Hepatitis C virus (HCV) is now established as the major parenteral type. However, more recently other related agents including hepatitis G virus have been identified [2]. This virus initiates infection by interacting with certain receptors on surfaces of susceptible cells [3,4] it infects the liver and replicate in the organ for many years. Transmission of HCV predominantly occurs parenterally as a result of blood transfusion and exposure to blood derivatives and the disease was first recognized in recipients of blood and blood products such as factor VIII and immunoglobulins. Transplanted organs and needles-stick injuries have also been implicated in transmission. Mc Lean *et al.* [2] reported transmission in drug misusers and patients in dialysis and surgical units. Sexual contact has also been incriminated in the transmission of HCV [5]. There is also a growing evidence of vertical transmission (mother to baby).

Hepatitis C virus has been shown to have a worldwide distribution, occurring among persons of all ages, genders, races and regions of the world [6].

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Recent report by the World health Organization (WHO) estimated that 170 million persons, or about 3% of the world's population, are infected with HCV who are at risk of developing liver cirrhosis, cancer or both [7]. Slightly different prevalence was reported from different regions of the world. Prevalence of 1.7% was reported from America, 1.03% from Europe, 3.9% from the Western Pacific, 4.6% from the Eastern Mediterranean, 2.15% from South Asia and 5.3% from Africa [7]. In Northern Europe, the prevalence of HCV infection among healthy blood donors varies between 0.01 and 0.02% [8-9] 6.5% in Equatorial Africa [10] and 20% in Egypt [11]. Here in Nigeria, available data showed that the prevalence of hepatitis C virus infection among local commercial blood donors ranged from 12.3-14.0% [9,12].

Various prevalence rates of anti-HCV antibodies have been documented in African countries. Prevalence rates reported from some African countries also differ from place to place, a low prevalence of 2.8% was found in blood donors in a Ghana study while 15.8% prevalence was reported among Egyptian blood donors [13,14]. Karuru *et al*. [15] reported 4.4% in Kenya; in 2004, Lassey *et al*. [16] recorded 2.5% in Ghana and 3.3% in Burkina Faso [17]. In Nigeria, the nation reported by Inyama *et al*. [18] as one of the countries highly endemic for viral hepatitis, the prevalence rate of HCV infection was earlier said to vary between 5.8% and 12.3% [12]. In tune with this, 5.8% prevalence was initially found among normal blood donors in Southern Nigeria [9] different states in Nigeria, such as Lagos, Osun and Plateau States have recorded anti-HCV antibody prevalence rates of 8.4% [19], 9.2% [20] and 5.7% [18] among blood donors, pregnant women and HIV patients respectively. However, Imoru *et al*. [21] reported HCV virus antibody prevalence of as low as 0.4% (n =2,288) among male blood donors in Kano State. The sero-prevalence of 6.0% in blood donors was also reported by Egah *et al.* [22] in Plateau State. With the low prevalence of anti-HCV in developed countries, the risk of infection is still estimated at about 1:100,000 [23]. This risk is expected to be higher in our environment where the prevalence is high in addition to the virtual non-existence of testing methods for HCV markers [22].

The differences in prevalence rates of anti-HCV antibodies between developed countries where prevalence rates are low and developing countries where prevalence rates are higher may be explained by certain factors. These include socio-cultural practices involving the use of sharp instruments contaminated by blood and body fluids for procedures such as scarifications, tattooing and circumcision and so on which are common practices in many developing countries [22]. Many of these countries also do not have facilities to test patients for hepatitis C virus infection. On the other hand, most developed countries are now having low prevalence rates of HCV because blood and blood products for transfusion are routinely tested for various blood-borne pathogens including HCV and measures such as the use of sterilized instruments and the needle exchange program for intravenous drug users has also served to reduce the prevalence of hepatitis C infection. In most developing countries, Nigeria inclusive, most blood transfusion units only test blood donors for hepatitis B virus antigen and the human immunodeficiency virus (HIV) antibodies [22].

However, in Nigeria, routine screening for HCV is yet to be practiced in most blood banks. The likely consequences of this are high transmission rate of this HCV through blood transfusion. Relatively, little work has been done in the area of establishing the prevalence of the HCV infection in the different subpopulations. In one of such works, Olubuyide *et al.* [24] working at the University College Hospital (UCH), Ibadan, estimates the anti-HCV antibodies among doctors and dentists at 11%. It is however desirable that studies on blood donors be done so that the current status of this viral subpopulation be known and the magnitude of the risk of transmission of HCV through blood transfusion can be highlighted for the attention of appropriate heath authorities. This study therefore, was designed to determine the prevalence of antibodies to HCV among blood donors in University College Hospital (UCH), Ibadan, to compare the seroprevalence determined with those reported for blood donors and other subpopulations in developed and other developing countries and to generate data which may be useful for appropriate health authorities.

MATERIALS AND METHODS

Subjects: The subjects included in this study were 200 (184 males and 16 females) blood donors in the blood bank of University College Hospital (UCH), Ibadan who consented voluntarily after thorough explanation of the purpose of the study.

Specimen Collection and Preparation: The collected patients' variables are age and gender. About 5 ml of

venous blood was collected from each donor into sterile bottles. The blood samples were left to clot, after which sera were separated from the clot by centrifuging at 2000 rpm for 10 minutes. Each serum sample was tested for the presence of antibody to HCV using test strips of a rapid, one step test kit (*RIGHTCHOICE* HCV, Israel) for the qualitative detection of antibodies to HCV in serum/plasma. The serum or plasma was separated from blood as soon as possible to avoid haemolysis. Only clear, non-haemolyzed specimens were used. Testing was performed immediately after the specimens were collected. Specimens were not left at room temperature for prolonged periods. Specimens not used immediately were stored at 2-8°C for up to 3 days. For long term storage, specimens were kept at below -20°C. The serum was then pipetted into sterile ependorf tubes and stored at -20°C until ready for use. All specimens were brought to room temperature prior testing. Frozen specimens were completely thawed and well mixed prior to testing and specimens were not frozen and thawed repeatedly.

Assay for HCV Antibodies: The test and interpretation of the results were done according to the guidelines of the kit's manufacturer. The assay was carried out using HCV serum/Plasma device developed by RIGHTCHOICE Diagnostics P.O. Box 360, Yavne 70650, Israel. The HCV Serum/Plasma Device is a rapid, one step test for the qualitative detection of antibodies to Hepatitis C virus (HCV) in serum or plasma. This device has relative sensitivity and specificity of > 99.0% and 98.6% respectively, with accuracy of 99.3%. The RIGHTCHOICE HCV serum/plasma device uses the principle of membrane based immunoassay for the detection of antibody to HCV in serum or plasma. The membrane is coated with recombinant HCV antigen on the test line region of each test device. During testing, the serum or plasma specimen reacts with the protein A coated particle. The mixture migrates upward on the membrane chromatographically by capillary action to react with recombinant HCV antigen on the membrane and generate a coloured line. Presence of the colored line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a coloured line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

Test Procedure: The test device, specimen and buffer were allowed to equilibrate to room temperature prior to the test. The test device was then removed from the foil pouch and placed on a clean and levelled working surface. The disposable specimen dropper was held vertically and specimen drawn in up to the fill line. It was transferred to the specimen well (s) of the test device. Two full drops of buffer were also added and the timer started. The result was read after 10 minutes. Two distinct red lines which appeared, one line in the control region (c) and another in the test region (T) indicates a positive reaction. One red line which appeared in the control region (c) with no other apparent red or pink line in the test region (T) was taken as a negative reaction while control line which failed to appear was taken as invalid reaction.

RESULTS

Of the total of 200 samples tested for HCV antibodies, 16 tested positive giving HCV antibody prevalence of 8.0%. Table 1 shows the seroprevalence of HCV antibodies in relation to the ages of the subjects. In the age group 30-39 years, a total of 78 samples were tested out which 9 tested positive thus, giving the highest seroprevalence of 11.5%. Age groups 20-29 and 40-49 years showed seroprevalence of 6.6% and 4.4% respectively while age group ≥ 50 years showed zero prevalence as shown in Table 1.

Table 2 shows the seroprevalence of HCV antibodies in relation to the sex of the subjects. The study group comprises of 184 male and 16 female blood donors. The overall seroprevalence of HCV antibodies in the study group is 8%. Fifteen (8.2%) out of the 184 males were seropositive for HCV antibodies while 1 (6.3%) out of 16 female blood donors was seropositive for HCV antibodies (Table 2).

Table 1: Distribution of anti-HCV Antibodies by Age Groups

Age Group (Years)	No. Tested	No. Positive (%)
20-29	91	6(6.6)
30-39	78	9(11.5)
40-49	23	1(4.43)
≥50	08	0(0.0)
Total	200	16(8.0)

Table 2: Distribution of anti-HCV Antibodies by Sex

Sex	No. Tested	No. Positive (%)
Male	184	15(8.2)
Female	16	1(6.3)
Total	200	16(8.0)

DISCUSSION

Hepatitis C virus is an important cause of morbidity and mortality. Detection of antibodies to various hepatitis C viral antigens indicates infection with the virus and in most cases portrays a chronic infection [2]. The course of the chronic hepatitis can be prolonged and insidious and infected persons may not develop symptoms for many years after onset of chronic infection [2]. Though, with the introduction of routine screening for hepatitis C in blood donors in the developed countries of the world, cases of post transfusion hepatitis have been greatly reduced [25], it is still high in developing countries such as Nigeria. However, during May 1990, routine testing of donors for evidence of HCV infection was initiated and during July 1992, more sensitive multiantigen testing was implemented, reducing further the risk for infection to 0.001% per unit transfused [23].

In this study, a prevalence of antibodies to HCV among blood donors in University College Hospital (UCH), Ibadan-Nigeria was found to be 8.0%. This rate (8.0%) is higher than the 3.0% worldwide seroprevalence reported by the World Health Organization (WHO) in 1999 [7]. The 8.0% seroprevalence obtained in this study is also higher than the 5.3 % reported for the whole African region by WHO [7]. However, this rate (8.0%) is slightly lower than the 12.3-14.0% range reported by Mutimer *et al*. [9] and Halim and Ajayi [12] among local commercial blood donors in Nigeria. It is however, higher than 3.0% (n=366) reported by Ejele *et al*. [25] in Niger Delta, Nigeria and less than 8.4% (n=167) seropositivity documented for blood donors in Lagos [19]. The relatively high prevalence observed in Nigeria and indeed this study could be attributed to a number of factors which include poor health seeking behaviour, low socioeconomic and educational levels of the people [26].

The 8.0% anti-HCV seroprevalence reported in this study was higher than the 6% reported by Egah *et al.* [22] among twelve blood donors who were all males. Consistent with the observation of Inyama *et al*. [18], males in this study had higher (8.2%) HCV antibody prevalence than the females (6.3%). This might be due, in part, to higher number of males that enrolled in the study. This observation is contrary to that of Ejele *et al*. [27] who reported that females had higher HCV antibody prevalence than males in Niger Delta, Nigeria. Statistical analysis, however, showed no significant difference ($p>0.05$) between the prevalence rates of the male and female individuals. Inyama *et al*. [18] and Mustapha *et al*. [28] made similar observations between male and female genders in Nigeria populations. In addition, though among sickle-cell anemic patients, Torres *et al*. [29] also observed no significant gender difference in HCV antibody prevalence in Brazilian population.

Analysis of the age related seroprevalence of HCV antibodies in this study showed that age group 30-39 years had the highest prevalence of 11.5 % followed by age group 20-29 years which had prevalence of 6.6 % while age group $\geq$ 50 years had zero prevalence. This slightly agrees with the pattern observed in the United States where highest prevalence was observed among persons 30-49 years old [5]. Another pattern that emerged was is observed in Egypt, where the prevalence of HCV infection increased steadily with age and high rates of infection are observed among persons in all age groups [30]. The findings of this study were also in agreement to observations of Ejele *et al*. [27] and Ayolabi *et al*. [19] who reported highest prevalence of HCV antibodies in the age group 30-39 years, the supposedly sexually active group. The reason for these observed differences in the prevalence pattern of HCV infection in different parts of the world is not immediately known to this study, but this was suggestive of the probability of transmission routes other than sexual as mode of acquisition of the HCV among the seropositive patients. There was, however, no statistical association ($p =0.05$) between age of the patients and prevalence of HCV antibodies.

Percutaneous exposures to infected blood and blood products were reported to be the major route of transmission of HCV. Cultural practices such as tattooing, ear piercing, circumcision, face marking (tribal marks) are widely practiced in underdeveloped countries including Nigeria [31]. Most of the times, these practices are carried out using unsterilized equipments such as blades and knives. Prior to the advent of HIV and AIDS in Nigeria, there was lack of enforcement of regulations guiding blood transfusion in many localities; this enhanced indiscriminate blood transfusion practices and the dominance of commercial donors among blood donors. According to the Federal Ministry of Health Nigeria [32], there was high patronage of patent medicine stores or some other substandard settings for treatment of ailments where unsterilized sharps were often used.

The presence of antibodies of various hepatitis antigens indicates infection with the virus and in most cases portrays a chronic infection [2]. The donors were apparently free of symptoms and were therefore deemed qualified to donate blood. This portends great risks to the general populace, as the transmission of HCV predominantly occurs parenterally as a result of blood

transfusion and exposure to blood derivatives. Patients transfused with blood from the seropositive donors are subject to direct transmission of the hepatitis C virus. The healthcare workers are also not left out of the risks. These include the laboratory scientists who bleed these donors, the doctors who transfuse the blood to the patients, the nurses who care for the patients as well as the surgeons and dentists who may have to operate on the patients. This partly explains why Olubuyide *et al*. [24] observed a high prevalence (11 %) of antibodies to HCV among doctors and dentists working at the University College Hospital (UCH), Ibadan-Nigeria.

In conclusion, our findings further confirm the presence of hepatitis C virus infection Nigeria [33,34, 22]. It has shown an HCV antibody prevalence of 8.0% among blood donors in the University College Hospital, Ibadan, Nigeria. This rate will have serious contribution to the morbidity and mortality. The finding of a high prevalence of HCV antibodies in blood donors in Ibadan brings to the fore the necessity of adopting measures that will ensure that blood is transfused to its recipients with minimal risk of transmission of HCV. Screening of blood donors for HCV antibodies is strongly recommended. There is an urgent need to introduce routine screening of blood donors for Hepatitis C virus markers in centers where this is not currently being practiced. This will reduce the risk of transfusion-associated hepatitis C virus infection and its complications in Nigeria [22]. Assay of HCV antibodies in other identifiable subpopulations like drug users, commercial sex workers should also be undertaken in order to ascertain the prevalence for these groups. Therefore, until vaccine is hopefully developed following successful *in vitro* culture of infectious HCV [35] behavioral attitudes that will lessen the risk of contracting HCV are recommended for the general population. Further research at the molecular level will reveal the predominant genotype in circulation which might aid in the development of appropriate vaccine.

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