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B. A. Abeni¹, N. Frank-Peterside¹, O. E. Agbagwa¹, S. A. Adewuyi¹, T. I. Cookey¹ and I. O. Okonko^{1*}

¹Virus Research Unit, Department of Microbiology, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors NFP, OEA, IOO and BAA designed the study, performed the statistical analysis and wrote the protocol. Authors IOO and TIC managed the analyses of the study. Authors IOO, BAA and SAA managed the literature searches and wrote the first draft of the manuscript. Authors NFP and OEA supervised the whole study which, author BAA used as part of her M.Sc. Dissertation in the Department of Microbiology, University of Port Harcourt, Nigeria. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The objective of this study was to determine the seroprevalence of hepatitis C virus (HCV) among intending blood donors at two Hospitals (government and private-owned) in Rivers State, Nigeria.

Study Design: Cross-sectional study.

Place and Duration of Study: Two Hospitals (government and private-owned) in Rivers State, Nigeria, between January 2018 and April 2019.

Methodology: Two hundred and eighty-two (185 males and 97 females, age groups 18-50 years old) blood donors were recruited for this study. Blood samples were screened for antibodies to Hepatitis C virus (HCV) using DiaSpot® HCV-Ab Test strips and enzyme-linked immunosorbent assay (ELISA) based kits, following the manufacturer's description.

Results: Of the 282 screened prospective donors (males and females) in this study, the total prevalence of HCV from both hospitals was 4.6% with a seronegativity was 95.4%. Male donors

*Corresponding author: Email: iheanyi.okonko@uniport.edu.ng;

had more cases of HCV (69.2%) than the females (30.8%) whereas the age group 21- 30 years had the highest prevalence of HCV (53.8%). Also, donors with tertiary and secondary education had a tie of (46.2%) as the highest prevalence rate of HCV, this was followed by those with primary education (7.7%). Unmarried donors had higher HCV prevalence (76.9%) compared to their married counterpart (23.1%). Meanwhile, family donors had the highest prevalence of HCV (61.5%) as compared to the relatively low prevalence among voluntary donors (7.7%) and paid donor (30.8%). Concerning occupation, students had the highest prevalence (46.2%), followed by unemployed donors (30.80%), business owners (15.4%) and lastly, civil servants (7.7%). The only significant relationship was found between HCV prevalence and HCV awareness (p=0.001), while the association with other demographic factors and HCV prevalence was not significant (p>0.05). **Conclusion:** The seropositivity of HCV among blood donors in Port Harcourt, Rivers State, Nigeria was low but remains a great danger to public health. Therefore, HCV screening by ELISA methods in all donors is recommended.

Keywords: Blood donors; HCV antibody; one step-rapid test; ELISA assay-based test; Rivers State; Nigeria.

1. INTRODUCTION

During a blood transfusion, various infectious agents are transmitted to the recipients and they regularly include viral agents. These agents oftentimes cause asymptomatic, acute, chronic and latent infections. Precautionary measures for safe blood transfusion involve detailed questioning of donors and screening tests. World Health Organization endorses screening of all donated blood for transfusion-transmitted infections (TTIs) like HCV, HBV, HIV and HTLV as an obligatory measure [1].

Hepatitis C virus (HCV), an RNA virus from the family Flaviviridae is transmitted particularly via parenteral route as well as vertically and sexually. HCV infection is clinically significant due to its persistence in approximately 85.0% of infected individuals, presenting a vital prospect of chronic liver damage [2].

Its endemicity has long been validated in Nigeria but there is less information about its epidemiology [3]. Various prevalence of HCV antibody among blood donors have been reported in the country, though less common among teenagers, they could benefit from this surveillance through early detection and treatment. Here, the objective of this study was to determine the seropositivity of HCV among intending blood donors at two hospitals in Rivers State, Nigeria and demographic distribution among sex, age, education level, marital state, occupation and awareness.

2. MATERIALS AND METHODS

2.1 Study Design

A cross-sectional study was carried out among blood donors at the University Port Harcourt

Teaching Hospital (UPTH), a government-owned hospital and Meridian hospital, a private-owned hospital, both in Rivers State, Nigeria from January 2018 and April 2019. A structured questionnaire was administered to consenting blood donors to obtain information on sociodemographic factors before sample collection.

2.2 Sample Size Determination

The sample size for this study was determined using the formula: $N=Z^2PQ/d^2$ [4]. Where N is the desired sample size, P is the expected prevalence in the target population, Q = 1-P, Z = 1.96 standard error, d is the level of statistical significance (0.05). A P-value of 2.9% (2.9% reported for HCV among blood donors in Rivers State as of 2005 by Koate et al. [5] and was used for representing maximum uncertainty.

Z= Normal standard deviation at 1.96 (standard error at 95%)

P= Prevalence of HCV (2.9% for blood donors in Rivers State as at 2005) = 0.029

q=1-p (1-0.029) = 0.971

d=degree of accuracy/precision expected=0.05 N= 43 (estimated).

Hence, the estimated sample size was 43 with an additional 10% sampled (4.3) to take care of data inconsistencies [6], providing a total sample size of 47.3 approximately a minimum of 50 samples for the study.

2.3 Study Population

A sum of 282 blood donors (185 males and 97 females) were recruited for this study. Both males and female were included in the study. Underage individuals were, however, excluded.

The age group of the donors were 18 - 50 years old. This was stratified into age groups $\leq 20, 21$ -30; 31-40 and 41-50 years). The donors were further stratified according to marital status (singles and married), educational background (primary, secondary and tertiary education), occupational risk (which include student, business, civil servants and unemployed), donor types (paid, family and voluntary) and their level of awareness. Before the recommendation of donors for blood donation, blood samples were screened for HCV by using standard guidelines. Potential donors who tested positive were referred for treatment.

2.4 Sample Collection

A sum of 282 intravenous blood samples was obtained aseptically using a 5-ml syringe and allowed to clot at room temperature in plain tubes. Serum specimens were separated by centrifugation at 3000rpm (resolution per minute) for 5 min. The sera were stored at -20°C and used for the serological analysis.

2.5 Laboratory Assay for Detection of Anti-HCV Antibody

The manufacturers' standard operating instruction was strictly followed for the performance of all the tests.

2.5.1 Rapid Assay for detection of anti-HCV antibody

DiaSpot® HCV-Ab Test strips (manufactured by DiaSpot Diagnostics, USA), Global® HCV-Ab Kit (manufactured by Global Diagnostics, USA) and IND® HCV-Ab kits (manufactured by INDR Diagnostica, USA) were used in a stepwise order for the detection of HCV in the blood. These methods which are immunochromatographic and qualitative, detect the presence of HCV in human blood and can be read in-vitro having more than 99.9% sensitivity and 98.6% specificity. The DiaSpot® HCV-Ab Test strips (serum/plasma) is a qualitative, 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 the strip. 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 coloured 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. The interpretation of test results was performed according to the manufacturer's specifications.

2.5.2 ELISA techniques for the detection of HCV

These samples were further tested using a thirdgeneration enzyme immunoassay for the detection of anti HCV antibodies. Serum antibodies against HCV antibodies were analyzed *in vitro* using a commercial kit (DIA.PRO Diagnostic Bioprobes, Milano, Italy) based Enzyme-linked Immunosorbent Assay (ELISA). The serologic test and interpretation of results were done according to instructions of the kit manufacturer. Optical signals generated in the microwells were read at 450 nm with an ELISA plate reader. The ELISA kit manufacturer provided the formula for calculating the cut-off OD450nm (OD of negative control plus 0.250) which we used as a threshold for determining the reactive and non-reactive serum samples.

2.6 Statistical Analysis

Data were analyzed using Microsoft Excel spreadsheet (Microsoft Corporation). The seroprevalence was calculated. Pearson's Chi-square test was used to establish relationships between demographic factors and HCV prevalence. The level of significance was set at P ≤ 0.05 .

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 General characteristics of donors

Of the 282 blood donors enrolled in this study, 182 (64.5%) were presented at the University of Port Harcourt Teaching Hospital (UPTH) and 100 (35.5%) were attendees of Meridian Hospital, both in Rivers State, Nigeria. Table 1 shows the socio-demographical characteristics of the intending blood donors in both hospitals.

3.1.2 The overall prevalence of HCV

A total of 282 intending blood donors were screened for HCV. The overall demographic factors obtained from both hospitals and their statistical significance are shown in Table 2. When the prevalence of HCV infections was analyzed by gender, it was more common in men than in women (5.0% vs. 4.1%, p = 0.131).

Concerning age, HCV was most common among those aged 21-30 years, as compared to the older subjects (8.4% vs. 0.0%, p = 0.099) (Table 2). Among the unmarried blood donors, HCV prevalence was significantly greater than in married blood donors (6.0% vs. 3.0% (p = 0.228).

In terms of the level of education, donors with primary education had HCV prevalence more common than those with tertiary and secondary levels of education, respectively (10.0%, 5.0% vs. 4.2%, (p= 0.699). HCV prevalence was not significantly (p= 0.444) higher among students (7.0%) and unemployed donors (6.2%) as compared to the civil servant (2.4%) and business owners (2.3%) occupations (Table 2).

Besides, family donors had higher HCV positive than paid and voluntary donors (6.0%, 4.4% vs. 2.0% respectively, p=0.476). Finally, HCV infection was more common among patients who were not aware of the virus as compared with those who had heard of it (5.0% vs. 4.5%, p = 0.001) (Table 2).

3.1.3 Prevalence of HCV among blood donors in the comparison between the two hospitals

Of the 13 (5.0%) HCV-seropositive intending blood donors, 10 (5.5%) were from UPTH and 3 (3.0%) from Meridian hospital. The distribution of HCV prevalence follows the same patterns as observed among blood donors in general (Table 3).

3.2 Discussion

Of the 282 individuals screened, a total of 13 blood donors had positive HCV results. Overall prevalence was 5.0% for HCV antibodies. This was in line with 4.8% obtained by Halim and Ajayi [7]. This report is higher than 2.9% previously reported in Rivers State [5], and the 3.0%, 3.4% and 4.1% reported by Ejele et al. [8] in the Niger Delta region, in Kano State [9] and Benue State [10]. It was also higher than 3.2% and 4.4% obtained in Kenya and Ghana by Kamande et al. [11] and Walana et al. [12] respectively, when compared to other African countries. However, it was lower than 6.1%, 6.0%, 8.4%, 14.9%, 11.9%, 8.6% and 20% reported by Dammulak et al. [13] in Jos, Buseri et al. [14] in Osogbo, Ayolabi et al. [15] in Lagos, Ebie and Pela [16] in Enugu,

Table 1. Socio-demographical characteristics of the intending blood donors of both Hospitals

Demographic factors	Categories	No. Tested (%)	UPTH (%)	Meridian (%)
Gender	Males	185 (65.6)	125 (67.6)	60 (32.4)
	Females	97(34.4)	57 (58.8)	40 (41.2)
Age (years)	< 20	92 (32.6)	83 (90.2)	9 (9.8)
	21 – 30	83 (29.4)	46 (55.4)	37 (44.6)
	31 – 40	75 (26.6)	35 (46.7)	40 (53.3)
	41 - 50	32 (11.3)	18 (56.3)	14 (43.7)
Marital status	Single	172 (61.0)	126 (73.3)	46(26.7)
	Married	110 (39.0)	56 (50.9)	54 (49.1)
Educational level	Primary	10 (3.5)	7 (70.0)	3 (30.0)
	Secondary	143 (50.7)	91 (63.6)	52 (36.4)
	Tertiary	129 (45.7)	84 (65.1)	45 (34.9)
Occupational risk	Student	90 (31.9)	67 (74.4)	23 (25.6)
	Business	86 (30.5)	44 (51.2)	42 (48.8)
	Civil servants	41 (14.5)	20 (48.8)	21 (51.2)
	Unemployed	65 (23.0)	51 (78.5)	14 (21.5)
Donor types	Paid	91 (32.3)	67 (73.6)	24 (26.4)
-	Family	136 (48.2)	81 (59.6)	55 (40.4)
	Voluntary	55 (19.5)	33 (60.0)	22 (40.0)
Awareness	Yes	88(31.2)	58 (66.0)	30 (34.0)
	No	194 (68.8)	124 (63.9)	70 (36.1)
Total		282 (100.0)	182 (64.5)	100 (35.5)

Demographic factors	Categories	No. Tested (%)	No. Positive (%)	p value
Gender	Males	185 (65.6)	9 (5.0)	
	Females	97(34.4)	4 (4.1)	P= 0.131
Age (years)	< 20	92 (32.6)	5 (5.4)	
	21 – 30	83 (29.4)	7 (8.4)	
	31 – 40	75 (26.6)	1 (1.3)	
	41 - 50	32 (11.3)	0 (0.0)	P = 0.099
Marital status	Single	172 (61.0)	10 (6.0)	
	Married	110 (39.0)	3 (3.0)	p = 0.228
Educational level	Primary	10 (3.5)	1 (10.0)	
	Secondary	143 (50.7)	6 (4.2)	
	Tertiary	129 (45.7)	6 (5.0)	p= 0.699
Occupational risk	Student	90 (31.9)	6 (7.0)	
	Business	86 (30.5)	2 (2.3)	
	Civil servants	41 (14.5)	1 (2.4)	p = 0.444
	Unemployed	65 (23.0)	4 (6.2)	
Donor types	Paid	91 (32.3)	4 (4.4)	
	Family	136 (48.2)	8 (6.0)	
	Voluntary	55 (19.5)	1 (2.0)	p = 0.476
Awareness	Yes	88(31.2)	4 (4.5)	
	No	194 (68.8)	9 (5.0)	P= 0.001
Total		282 (100.0)	13(5.0)	

Table 2. Summary of the relationship between the overall HCV prevalence concerning the demographic factors of the intending blood donors of both Hospitals

Table 3. HCV prevalence among intending blood donors about the two hospitals

Demographic factors	Categories	UPTH	No. positive (%)	Meridian	No. Positive (%)
Gender	Males	125 (67.6)	7(5.6)	60 (32.4)	2(3.3)
	Females	57 (58.8)	3(5.3)	40 (41.2)	1(2.5)
Age (years)	< 20	83 (90.2)	5(6.0)	9 (9.8)	0(0.0)
	21 – 30	46 (55.4)	5(11.0)	37 (44.6)	2(5.4)
	31 – 40	35 (46.7)	0(0.0)	40 (53.3)	1(2.5)
	41 - 50	18 (56.3)	0(0.0)	14 (43.7)	0(0.0)
Marital status	Single	126 (73.3)	7(5.6)	46(26.7)	3(6.5)
	Married	56 (50.9)	3(5.4)	54 (49.1)	0(0.0)
Educational level	Primary	7 (70.0)	0(0.0)	3 (30.0)	1(33.3)
	Secondary	91 (63.6)	6(6.6)	52 (36.4)	0(0.0)
	Tertiary	84 (65.1)	4(4.8)	45 (34.9)	2(4.4)
Occupational risk	Student	67 (74.4)	5(7.5)	23 (25.6)	1(4.4)
	Business	44 (51.2)	1(2.3)	42 (48.8)	1(2.4)
	Civil	20 (48.8)	1(5.0)	21 (51.2)	0(0.0)
	servants				
	Unemployed	51 (78.5)	3(5.9)	14 (21.5)	1(7.1)
Donor types	Paid	67 (73.6)	0(0.0)	24 (26.4)	2(8.3)
	Family	81 (59.6)	8(9.9)	55 (40.4)	1(1.8)
	Voluntary	33 (60.0)	2(6.1)	22 (40.0)	0(0.0)
Awareness	Yes	58 (66.0)	4(6.9)	30 (34.0)	0(0.0)
	No	124 (63.9)	6(4.8)	70 (36.1)	3(4.3)
Total		182 (64.5)	10(5.5)	100 (35.5)	3(3.0)

Strickland [17] in Kaduna, Natalie et al. [18] in Burkina Faso and Frank et al.[19] in Egypt, respectively. Higher frequency of HCV was found in males. Though gender association was not statistically significant, it can be said that more male tested positive to HCV due to the uneven distribution of donors in terms of gender. There was a predominance of males among the study participants because females are not encouraged to donate blood in Nigeria society. This is in line with previous studies elsewhere in Nigeria. Okonko et al. [20] reported anti-HCV antibody solely among male blood donors in Ibadan, Nigeria. Egah et al. [21] also reported anti-HCV positivity among male blood donors in Jos, Nigeria. Our study agrees with that of Buseri et al. [14] in which the anti-HCV positive blood donors were majorly male than females in Osogbo, Nigeria. This demographic pattern has been replicated in earlier studies from other parts of Nigeria [22-24].

The 282 donors recruited in the study were within ages ranging from less than 20 years to 50 years. In general blood, donors must fulfil certain requirements, one of which is the age requirement of between 18 - 60 years. Nevertheless, most youths within ages 18-30 donate blood mostly for incentive purposes as a way of earning extra money. Even though there were many (n=92) donors within the less than 20 age group in this study, the 21-30 years' age group had the highest prevalence of HCV (8.4%). The high prevalence within this group can be explained by some careless social and sexual activities this group sometimes exhibit. This finding also deviated from that of Okonko et al. [20] who reported a higher prevalence of HCV in blood donors aged 40 years and above in Ibadan, Nigeria.

Prevalence of antibody to HCV (6.0%) in this study was significantly higher among the unmarried participants than in married (3.0%) individuals. This correlates with the study of Afolabi et al. [25] in Ibadan who also reported a higher HCV prevalence among the unmarried group. Higher prevalence values of HCV antibody in the unmarried group maybe because the unmarried individuals have a higher tendency of engaging in illicit behaviours putting them at higher risk of contracting HCV and other infections.

About occupation, higher frequency of HCV was recorded among students (7.0%) and the lowest was in business owners (2.3%) respectively. No significant relationship was found in this regard, the cause of this occupation-related predisposition is unknown. It could be as a result of poverty or low socio-economic standing which might have compelled some donors to indulge in risky alternatives (to survive) that could predispose them to this virus and their living environment, unlike those of middle to high financial standards.

As regards to the awareness of the donors about HCV infection, the majority of donors claimed not to have heard of HCV before donation. A significant association (p=0.001) was found to exist between HCV and awareness, as most participants that tested positive to HCV (5.0%) fell under those that knew nothing of the pathogen.

This study identified family donors as the dominating donor type, followed by the paid donors, while the voluntary donors were the least in number. This result, however, does not correspond to the findings of previous reports of Buseri et al. [14] and Okocha et al. [22] which recorded a higher number of commercial donors as compared to family donors. Family blood donors recorded the highest prevalence of HCV, while voluntary donors had the least. This further validates the earlier suggestion by World Health Organization (WHO) that family and paid donors are more likely to transmit transfusion transmissible infections than their voluntary counterparts [26]. A family member will most likely be unaware of his/her status until a need arises, such as family replacement blood donation. Furthermore, an individual desperate for finance will most likely hide their health status especially when the monetary reward is attractive. The amount of voluntarily donated blood has continued to fall over the years in Nigeria due to logistics and organizational problems associated with the national blood transfusion service and the orientation of the citizens.

The difference in the HCV prevalence of both hospitals was because of the different sample sizes obtained from the hospitals and could be as a result of the different socio-economic status of the people attending these facilities. The findings of this study showed that the hepatitis C virus is circulating in Rivers State, Nigeria and infecting the residents of the city, including the unaware blood recipients and blood donors. The results of this study reinforce the need for screening for transfusionmandatory transmissible infections (HIV, HBV, HCV and syphilis) in blood donations.

4. CONCLUSION

Hepatitis C virus seroprevalence was detected in 5.0% of blood donors. Though prevalence rates of various authors differ, these differences may

be due to geographical distribution, health practices among the study population, length of time studies were performed and age range of the studied population. On demographic relationship with the screening results, a significant association was found between HCV and awareness. All the other demographics had no significant association. Therefore, HCV screening by ELISA methods in all donors is recommended.

CONSENT

Informed and written consent was collected from the participants and were preserved by the author.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Hospital Research Ethics committees of University of Port Harcourt Teaching Hospital (UPTH) and Meridian Hospital and have, therefore, been performed following the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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