



## Original article

# Detection of hepatitis C virus antibodies among healthy blood donors at a tertiary hospital in Nigeria

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## ABSTRACT

**Background:** Most blood transfusion centres in Nigeria including the University of Abuja Teaching Hospital (UATH), Gwagwalada, Abuja use rapid test kit for screening blood donors. Though it is simple, robust with speedy results, it has some limitations. This cross-sectional study determined the prevalence of hepatitis C virus (HCV) antibodies by the currently used rapid detection assay among blood donors tested positive by enzyme-linked immunosorbent assay (ELISA) and described the HCV risk factors and socio-demographic characters among the blood donor participants. **Methods:** Blood samples were collected from 365 blood donors attending UATH blood bank, screened for HCV antibodies using ELISA kit (CTK Biotech, USA). Positive samples were retested using Aria rapid test kit. Structured questionnaires were used to collate subjects' socio-demographic data and risk factors of infection. **Results:** Out of 365 blood donors tested, 55 (15.1%) were positive for HCV antibodies. Of the 55 ELISA positive samples, 9(16.4%) samples were negative by Aria rapid test kit. Sociodemographic data showed that females had a higher prevalence (16.3%) than males (14.9%) ( $p = 0.81$ ). The age group with the highest rate of infection was 31-45 years ( $p < 0.0001$ ). Marital status of the blood donors was significantly associated with HCV seropositivity, as singles were more infected than married blood donors ( $p < 0.0001$ ). The most predictive risk factors associated with the HCV infection among blood donors at UATH included lack of knowledge of HCV, sharp and needle injury and tribal marks. **Conclusion/Recommendations:** The study revealed the superiority of ELISA over Aria rapid test kit in the detection of HCV antibodies. The missed positive cases by the currently used rapid test could pose a risk to blood transfusion safety and necessitates for preventive measures to be intensified, as blood donors with high risk factors be deferred from blood donation to reduce HCV transfusion risk in Nigeria.

## Introduction

Screening for transfusion-transmissible infections (TTIs) to exclude blood donations at risk of transmitted infection from donor to recipients is a critical part of the process of ensuring that transfusion is as safe as possible [1]. Effective screening for evidence of the presence of the most common and dangerous TTIs like HCV antibodies can reduce the risk of transmission to very low levels [2, 3].

Hepatitis C virus, one of the TTIs can cause serious public health problems in developing countries

due to its ability to develop chronic infections and most of the times it is asymptomatic [4]. Hepatitis C virus causes acute and chronic hepatitis which can eventually lead to permanent liver damage and hepatocellular carcinoma [5]. Hepatitis C virus affects about 180 million people worldwide and above 3-4 million people are infected with HCV every year [6]. So far the prevalence of HCV is increasing in Nigeria ranging from 4.7-5% in Ilorin to 5.3-6.6% in Enugu, to 11% in Ibadan and 20% in Benin [7]. Also in a study

conducted among university students in South Western Nigeria shows a prevalence of 0.7% HCV antibody using LabAcon which is lower than what has been recorded in some parts of Nigeria [8]. This shows that immune-chromatographic methods such as LabAcon rapid test kits are not sensitive enough to detect HCV status in all cases [9]. For example, higher HCV prevalence has been reported in South-East Asian countries including India (1.5%), Malasia (2.3%), Philippines (2.3%), Pakistan (8.1%), Egypt is as high as 20% [10]. Developed countries like Europe have a low prevalence like in Northern Europe the prevalence is between 0.01- 0.02% and in Southern Europe, they have a prevalence of 1-1.5% [11]. In contrast, HCV infection is higher among the developing countries, because of problems such as low quality in screening kits, unsafe medical practices and intravenous drug abuse with a shared needle [2].

At the University of Abuja Teaching Hospital, Gwagwalada, Abuja, Aria rapid diagnostic tests (RDT) device, is being used for the screening of blood donors in the blood bank. Other blood banks in Abuja and environs use different brands of the rapid test device and blood units are being sourced from those private medical laboratories for patients at the University of Abuja Teaching Hospital, Gwagwalada, Abuja. Recently, there are a lot of complaints of some prospective donors who tested positive with RDT kit presently in use, and when retested with another RDT, ELISA or the PCR will turn out negative. Also, most studies done in Nigeria on HCV antibodies used rapid test kits and this could be the reason for low prevalence rates obtained. It is on these facts that this study was conceived and designed to determine the prevalence of HCV antibodies by the currently used rapid detection assay among blood donors tested positive by ELISA.

The study also assessed the risk factors associated with HCV infection and investigated the sociodemographic variables among the blood donor participants. We anticipate that the result of this study will highlight the necessity for a change of screening method to improve service rendered to humanity and reduce transfusion transmissible HCV infection among blood recipients attending the University of Abuja Teaching Hospital blood bank. Besides, the data derived from this study will add to the knowledge of the national and regional scientific societies of the actual burden of HCV infection in Nigeria.

## Material and methods

### Study site, design and population

This cross-sectional study was done at the University of Abuja Teaching Hospital (UATH) Gwagwalada, Abuja, Nigeria. Healthy blood donors attending the

University of Abuja Teaching Hospital Blood bank from 1<sup>st</sup> August 2019 to 31<sup>st</sup> January 2020, were enrolled for the study.

### Ethical considerations, inclusion and exclusion criteria

Ethical clearance was obtained from the ethical committee of University of Abuja Teaching Hospital Gwagwalada, Abuja with Protocol number: UATH/HREC/PR/2017/012/122 and approval number UATH/HREC/PR/2017/012/008. Informed consent was obtained from all participants before sample collection. All donors who gave their informed consent were included as participants in this study.

### Sample size determination

A total of 365 healthy blood donors participated in this study. This was obtained by calculation using the Fisher formula as described by Araoye [12]. The formula is  $n = Z^2 p (1-p) / d^2$  Where  $n$  = required sample size,  $z$  = confidence level 95% (Standard value of 1.96).  $P$  = estimated prevalence of 20% as reported in Nigeria by Ejiofor et al [7].  $d$  = margin of error at 5% (standard value is 0.05). Sample size calculation performed obtained 246 blood donors. Finally, the sample size of 365 blood donors was recruited and enrolled in the study.

### Sociodemographic data and risk factors of HCV infection

The questionnaire was designed and used for the collection of sociodemographic data and risk factors like age, gender, marital status, occupation/profession, academic status number of sex partners, history of sexually transmitted diseases, transfusion history, domestic sharp/needle injury, alcohol/drug abuse etc. The questionnaire was filled by the participants and few participants were assisted by trained research assistants in reading to complete the questionnaire which didn't affect their answers.

### Specimen collection and processing

Five (5) ml of blood was collected by vein-puncture into plain test tubes and ethylene diamino tetra-amino acid (EDTA) containers. The samples in plain tubes were allowed to clot and thereafter centrifuged at 3000g for 5 minutes to separate the sera. Sera were separated into cryovials labelled and stored at -20°C until testing was performed.

### Assay Procedures

#### ▪ Determination of ABO blood group

ABO blood group of all blood donor participants were determined using cell grouping method as described in the American Association of Blood Bank (AABB) technical manual [13]. Anti A, anti-B and anti-D

reagents from Spectrum Biotech, USA was used following manufacturer's instructions. The principle of the ABO blood group test is based on antigen and antibody reaction resulting in agglutination.

▪ **Determination of HCV Antibodies using RecombiLISA test kit**

*Principle of the Assay*

The RecombiLISA HCV Ab ELISA test is a solid-phase ELISA based on the principle of the indirect enzyme immunoassay technique for the detection of the IgG and IgM to HCV in human serum/plasma. The RecombiLISA HCV Ab ELISA test is composed of two key components (1) Solid microwells pre-coated with recombinant HCV structure and non-structure antigens. (2) Liquid conjugate composed of protein A conjugated with horseradish peroxidase (HRP-Protein A conjugate). During the assay, the test specimen was first incubated with the coated microwells. The anti-HCV antibodies, either IgG and /or IgM, if present in the specimen binds to antigen coated on the microwell surface and any unbound specimen was then removed by a wash step. During second incubation with the HRP-Protein A conjugates, the anti-HCV antibodies absorbed on the surface of microwell react to the HRP-protein A conjugate forming a conjugate complex. Unbound conjugates were then removed by washing. After addition of the TMB substrate, the presence of the conjugate complex is shown by a blue colour resulting from a reaction between the enzyme and substrate. The reaction is then stopped by the addition of the stop solution and the absorbance value for each microwell is determined using a spectrophotometer at 450/630nm.

*The test procedure is as follows:*

The step by step procedure was strictly followed as in the manufacturer's insert. The absorbance was read using a STAT FAX 2100 PLATE READER at 450nm. The results were calculated using the formula as prescribed by the manufacturer. The cut-off value was calculated as stated by the kit producer. Cut-off value =  $0.15 + N$  where N is mean OD of the negative control. Specimen OD ratio = Specimen OD/cut off value. The result is interpreted as negative when specimen OD ratio is  $< 1.00$  and positive  $> 1.00$ . The negative result indicates that there are no detectable anti HCV antibodies in the specimen.

▪ **Detection of HCV Antibodies on ELISA positive samples using Aria rapid test kit**

All samples positive for HCV antibodies by ELISA were retested with ARIA rapid test kit used for screening blood donors at UATH Gwagwalada Abuja. The standard operating procedures and manufacturer's instructions were strictly followed.

*Principle of Aria rapid test kit*

This is a double antigen lateral flow chromatographic immunoassay. The test strip of 1) a burgundy coloured conjugate pad containing recombinant HCV fusion antigen (Core, NS3, NS4 and NS5) conjugated with colloidal gold, (HCV Ag conjugates) and a control antibody conjugated with colloidal gold 2) a nitrocellulose membrane strip containing a test line and a control line. The test line is pre-coated with recombinant HCV fusion antigen and the control line is coated with a control line antibody. The antibody to HCV if present in the specimen added will bind to the HCV antigen conjugate. The Immunocomplex is then captured on the membrane by the pre-coated non-conjugated V fusion antigen forming a burgundy coloured T-line, indicating a positive result. Absence of the T-line suggests a negative result. The test contains an internal control (C-line) which should exhibit a burgundy coloured line of the immunocomplex of control antibodies regardless of the colour development on T-line. If the C-line does not develop, the test result is invalid, and the specimen must be retested. Also, in-house negative and positive sera were used to validate the test kits.

**Statistical Analysis**

The generated data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0 for windows (Inc., Chicago, IL). Differences in proportions were compared using chi-square. Odds ratio (OR) was used to measure the risk factors independently associated with HCV transmission and computed employing logistic regression at 95% confidence level.

**Results**

**Seroprevalence of HCV Antibodies among healthy blood donors**

The prevalence of HCV antibodies among blood donors using CTK Anti-HCV ELISA kit was determined. Out of the 365 samples analyzed for HCV by ELISA, 55 samples were positive for HCV antibodies indicating 15.1% prevalence of HCV infection among blood donors attending the University of Abuja Teaching Hospital Blood Bank. The detection capacity of HCV antibodies on ELISA positive samples using Aria rapid test kit showed of the 55 positive samples by ELISA, 46 (83.6%) samples were positive by Aria rapid test kit device while 9 (16.4%) were negative.

**Risk factors of HCV transmission**

The risk factors associated with HCV infection among blood donors attending the University of Abuja Teaching Hospital blood bank were assessed. **Table 1,**

depicts the frequency of potential risk factors reported by the blood donors with HCV seroprevalence rates and the odds ratio (OR) estimated by univariate analysis. There was a statistically significant association of lack of knowledge of HCV with HCV seropositivity ( $p=0.04$ ), Domestic sharp/needle prick injury ( $p=0.03$ ) and tribal marks/tattoo ( $p=0.0001$ ).

#### Sociodemographic characteristics of blood donors

**Table 2** presents the demographic characteristics of blood donors with HCV infection attending the University of Abuja Teaching Hospital, Gwagwalada, Abuja. It was found that female blood donors were more infected with HCV than the male counterpart though there was no statistically significant difference  $P=0.81$ . The result also showed that males were more involved in blood donation than the females with a male-female ratio at 7:1.

The age group with the highest rate of HCV infection was 31-45 years. There is a statistically significant difference  $p<0.0001$ . Also, single blood donors (unmarried) were more infected with HCV than

married blood donors  $p<0.0001$ . Civil servants, farmers/artisans were more affected than individuals in other vocation, but there is no statistically significant difference associated with HCV infection among blood donors and occupation at the University of Abuja Teaching Hospital Gwagwalada, Abuja. Academic status has no association with HCV seropositivity and there was no hindrance to blood donation with academic attainment as all people of various academic status donated blood units for their patients.

#### ABO blood group of the participants

**Table 3** shows the ABO blood group of participants concerning HCV seropositivity. Blood group O had the highest participation in donation with 275 (75.3%) out of 365 blood donors that participated in the study. Whereas, blood group AB blood donors had the lowest attendance. There is no association of blood group with HCV seropositivity, though blood group B has the highest prevalence (29.6%).

**Table 1.** HCV infection among blood donors and potential risk factors of transmission.

Risk Factors	No. tested	No. (%) positive	OR	(95%CI)	P-value
<b>Lack of knowledge of HCV</b>					
Yes	205	25 (12.2)	0.56	0.31-0.99	0.04*
No	150	30 (20)			
<b>Domestic Sharp/Needle Prick Injury</b>					
Yes	112	10(8.9)	0.45	0.22-0.94	0.03*
No	253	45(17.8)			
<b>Previous Blood Transfusion</b>					
Yes	16	1(6.3)	0.36	0.05-2.81	0.333
No	349	54(15.5)			
<b>Multiple Sexual partners</b>					
Yes	6	0(0)	0.42	0.02-7.6	0.56
No	359	55(15.3)			
<b>History of Sexually Transmitted Diseases</b>					
Yes	23	0(0)	0.11	0.0-1.84	0.12
No	342	55(16.1)			
<b>Alcohol/Drug Abuse</b>					
Yes	29	2(6.9)	0.39	0.09-1.71	0.21
No	336	53(15.8)			
<b>Previous Surgery/Dialysis</b>					
Yes	6	0(0)	0.42	0.02-7.6	0.56
No	359	55(15.3)			
<b>Tribal Marks/Tatoo</b>					
Yes	39	15(38.5)	4.46	2.16-9.22	0.0001*
No	326	40(12.3)			

\*Statistically significant ( $P<0.05$ ).

**Table 2.** Seroprevalence of HCV antibodies by sociodemographic variables .

Blood donors demographics	No. of blood donors screened	No. (%) positive for HCV	Chi-Square
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	n= 365	n= 55	(p-value)
<b>Gender</b>			
Male	322	48 (14.9)	0.06
Female	43	7 (16.3)	(0.81)
<b>Age Group (Years)</b>			
18-30	186	14 (7.5)	23.2
31-45	152	39 (25.7)	(<0.0001)*
46-60	27	2 (7.4)	
<b>Academic Status</b>			
Primary	32	6 (18.8)	0.38
Secondary	120	18 (15.0)	(0.83)
Tertiary	213	31 (14.6)	
<b>Marital Status</b>			
Married	186	13 (6.99)	24.9
Single	167	42 (25.1)	(<0.0001)*
Separated	12	0 (0)	
<b>Occupation</b>			
Farmer/Artisans	60	12 (20.0)	
Students/Applicants	66	8 (12.1)	5.8
Health workers	31	5 (16.1)	(0.211)
Civil servants	94	21 (22.3)	
Business	84	9 (10.7)	

\*Statistically significant ( $P<0.05$ ).

**Table 3.** ABO blood group and HCV seropositivity.

Blood group	Total no. (%) of participants	No. positive for HCV (%)	Chi-Square (p-value)
<b>A</b>	54 (14.8)	8 (14.8)	4.97 (0.117)
<b>B</b>	28 (7.7)	8 (28.6)	
<b>AB</b>	8 (2.2)	0 (0)	
<b>O</b>	275 (75.3)	39 (14.2)	
<b>Total</b>	<b>365 (100)</b>	<b>55 (15.1)</b>	

\*Statistically significant ( $P<0.05$ ).

### Discussion

The findings from this study have clearly shown a 15.1% prevalence rate of HCV infection among healthy blood donors attending UATH, Abuja using HCV ELISA. This value is higher than the prevalence rates of 1.8% obtained by **Isa et al.** [14] at Ahmadu Bello University Teaching Hospital, Zaria, Nigeria and 3.1% obtained by **Damola et al.** [15] at Lagos, Nigeria. In another study in Jos, Nigeria, a prevalence of 6% of HCV antibodies among blood donors was obtained by **Egah** [16]. Also, another study on the prevalence of HCV antibodies among blood donors in South Eastern Nigeria found a prevalence of 7.6% [17]. While similar work is done

in Lagos reported an HCV antibody prevalence of 8.4% [18]. Different prevalence rates observed from various studies could be as a result of different prevalence in a different area, kit potency, configuration and analytical sensitivity and specificity of the various test kit used in testing [19]. In this study, ELISA was used which has a higher sensitivity than rapid test device used in most areas with low prevalence rates. Moreover, poor storage of these rapid test devices could affect the potency which could affect the sensitivity, resulting in false results [19].

The ELISA result comparing it with rapid test kit understudy collaborated with a similar work done by **Osuji et al.** [1] at two tertiary health institutions in Nigeria. They obtained an 8.1% rate of HCV infection

among blood donors that tested negative using CTK Biotech rapid anti-HCV kit. Although there was no statistically significant difference comparing Aria rapid test kit and ELISA, Aria rapid test kit gave 16.4% rate of a false negative. This means that there is a 16% probability of transfusion of HCV infection using Aria rapid test kit in screening blood donors for HCV infection. This signified that ELISA 4<sup>th</sup> generation kit is more sensitive and superior than Aria rapid test devices in the detection of HCV infection among blood donors at UATH. Failure of the rapid kits to detect the presence of anti-HCV may be due to inadequate coating of the antigen, nature of antigens used and genetic heterogeneity of the virus [19].

Hepatitis C virus risk factors assessment among blood donors attending UATH Abuja was performed. This result showed that out of 365 blood donor participants tested, 205 blood donors lacked knowledge of HCV (had not heard of HCV and its modes of transmission), 25 representing 12.2% were infected with HCV. Another risk factor that showed a high rate of HCV infection was blood donors with tribal marks with 15 (28.3%) of 39 blood donors assessed had tribal marks and were infected with HCV. This study observed a statistically significant difference among blood donors with HCV infection concerning domestic needle/sharp injuries. The significant and predictive risk factors among blood donors in the study population include lack of knowledge of HCV infection, domestic needle/sharp injuries and possession of tribal marks. This study collaborated with the study of **Mitrovic et al.** [20] that recorded that tattooing as a significant risk factor associated with HCV infection among blood donors in Serbia and the work of **Damola et al.** [15] that observed that sharing of sharps for tattoo/tribal markings had a significant association with HCV seropositivity among blood donors in Lagos, Nigeria but disagrees with a study in Georgia USA by **Zaller et al.** [21] that reported that five risk factors remained independently predictive of HCV among blood donors including previous intravenous drug use, having lived in prison or juvenile detention centre, previous blood transfusion, sexual contact with intravenous drug user and tattooing. They concluded that most blood donors acquired HCV infection by percutaneous exposure to contaminated blood. Although there was no significant association of HCV seropositivity with the risk factors such multiple sexual partners, history of sexual transmitted diseases and alcohol/drug abuse (**Table 1**) in the study population, these risk factors are critical in HCV transmission and could be classified as

deferral criteria for blood transfusion. Also, many blood donors may not disclose some of these risk factors like multiple sexual partners and history of sexually transmitted diseases. This could be the reason of low values recorded.

Sociodemographic analysis of data obtained from this study showed that most of the blood donors infected with HCV infection at the University of Abuja Teaching Hospital are within age 18-45 years old with median and mean age as 32 years and 31.4 years respectively. This disagrees with the study of **Damola et al.** [15] that found blood donors  $\geq 50$  years had the highest prevalence of HCV infection in Lagos, Nigeria. The age bracket (31-45 years) is the most sexually active age as HCV is sexually transmitted [22]. This study found a statistically significant association between HCV infection and the variables tested such as age and marital status but not with gender and occupation/profession. Females are more infected than males but there is no statistically significant difference. This also collaborates with the work of **Damola et al.** [15]. The singles are more infected than the married blood donors and this agrees with the study of **Damola et al.** [15]. There is need therefore for people to have adequate knowledge of the infection and mode of transmission so that they can take proper precautions to prevent infection transfusion transmissible viral infections particularly HCV infection. The ABO blood group of the blood donors was studied about HCV seropositivity. The finding from this study showed that blood group O is the most blood units donated at UATH Abuja with about 75% of participants. This could be that blood group O is a universal donor. The study did not find an association of blood group with HCV seropositivity. This means any person can be infected irrespective of the blood group he belonged. This agrees with the study of **Osuji et al.** [23] that recorded high attendance of blood group O (73.6%) in a study at two tertiary hospitals in Nigeria.

### Conclusion

The prevalence of HCV antibodies among healthy blood donors at the University of Abuja Teaching Hospital Abuja was 15.1% using ELISA technique. The study revealed the superiority of ELISA over Aria rapid test kit in the detection of HCV antibodies. The missed positive cases by the currently used Aria rapid test can have catastrophic consequences in transfusion safety and can be minimized as blood donors with high risk factors be deferred from blood donation. Health education program on preventive and control measures should be

directed toward increasing knowledge of HCV infection to the populace and precautions taken in handling sharps. Also, tribal markers/tattoo facilities and equipment should be sterilized. The study recommends changes in the national policy for selection of blood donors so that strict deferral criteria are followed until shifting to the use of more sensitive and specific assays becomes feasible. Future studies on a wider scale should be encouraged particularly on the determination of performance characteristics of rapid test kits to evaluate the test kit with a comparable sensitivity, specificity and accuracy with ELISA kit.

#### Limitations of the study

Lack of financial support stuck the examination of blood donors tested negative by ELISA by the rapid test. Thus we couldn't calculate the rapid test accuracy including sensitivity and specificity.

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#### Data Availability

The data used to support the findings of this study are included in the article. The raw data of this study will be made available on request. All requests should be made to the corresponding author of this article.

**Conflict of interests** :No conflict of interest

#### Authors' contributions

This project was carried out in collaboration among the authors. The study was conceived and designed by GCD. GCD recruited the blood donors and ensured that samples were collected and preserved. AIO and GCD performed laboratory testing. AIO analyzed the data and wrote the draft manuscript. GCD and HK provided ideas and useful comments during manuscript preparation. All the authors read and approved the final manuscript.

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