

*Full Length Research Paper*

# **Sero-prevalence study of Hepatitis C virus infection among blood donors attending selected blood banks in some Local Government Areas in Kano, Nigeria**

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**Hepatitis C virus (HCV) infection afflicts more than 170 million people worldwide, with the great majority of patients having acute hepatitis C later developing into chronic HCV infection. 320 participants (80 in each of four blood banks from Wudil, Gaya, Sumaila and Takai Local Government Areas (LGA)) were tested for antibodies to HCV. Out of a total of the 320 subjects, 319 were males and 1 female between the ages of 14 to 54 years. Third-generation enzyme-linked immunosorbent assay was used in the analysis. 16 out of the 320 were reactive, representing 5.0% of the total population studied. This showed a high prevalence of HCV infection among blood donors in this part of the country when compared with those established for western countries. Prevalence rate based on the 80 subjects in each LGA indicated Gaya and Wudil to have highest prevalence of 5(6.3%) each, followed by Sumaila with 4(5.0%). Takai was observed to have the least prevalence rate of 2(2.5%). The relationship between the demographic data with HCV infection revealed no statistical significance ( $P > 0.05$ ). However, the relationship between possible risk factors and HCV infection revealed significant statistical association in the transmission through family (vertical transmission), receipt of injection as well as consumption of alcohol. Other possible risk factors such as blood transfusion did not reveal statistical association even though there were differences in positivity across the risk factors.**

**Key words:** Seroprevalence, Hepatitis C virus, Blood Banks, Kano.

## **INTRODUCTION**

Hepatitis C virus (HCV) infection afflicts more than 170 million people worldwide, with the great majority of patients with acute hepatitis C developing chronic HCV infection. It can ultimately result in liver cirrhosis, hepatic failure or hepato-cellular carcinoma, which is responsible for hundreds of thousands of deaths each year. Hepatitis C virus is a positive stranded RNA virus classified as a separate genus *Hepacivirus* in the family *Flaviviridae* (Jawetz and Adelberg, 2007). The HCV appears to have a narrow host range (Rogo, 2011). Human and

chimpanzees are the only known species susceptible to infection, with both species developing similar disease. According to Lansing and Donald, (2000), the structure and replication of HCV are incompletely understood due to low viral titers found in sera and livers of individuals infected and lack of an efficient cell culture system or small animal model permission for HCV infection. But with heterologous expression systems, functional CDNA (complementary DNA) clones, and most recently, selectable sub genomic replicons have been made (Choo et al., 1989; Rogo, 2011). Several different genotypes of HCV with slightly different genomic sequences have since been identified that correlate with differences in response to treatment with interferon (Howard, 2002; Rogo, 2011). Within an infected individual, HCV consists

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of a population of closely-related but heterogeneous sequences, called quasispecies that result from rapid development of mutations in critical region of the envelope protein (Farci, 1991). The HCV has long incubation period of 2 to 26 weeks (Chukwurah et al., 2005). The virus is plasma-borne and has the same routes of transmission in common with hepatitis B virus—sexual contact, exposure to contaminated blood and blood products, or vertical transmission that is from mother to her fetus during the prenatal period. HCV infection is usually silent and acute phase is asymptomatic, progressing to chronic cirrhosis and hepatocellular carcinoma. Hepatitis due to HCV is quite similar to hepatitis due to other agents but the disease is commonly without jaundice and often clinically mild (Jawetz and Adelberg, 2007). Primarily, liver cells damage coincides with the development of the host immune response and not with infection and viral replication. In addition, persistent viral replication often occurs without evidence of liver damage, suggesting that HCV is not directly cytopathic (Darius et al., 2001). The symptoms includes decreased appetite, fatigue, abdominal pain, occasional jaundice, itching, malaise, raised serum transferase level, muscle pain, joint pain, sleep disturbance, diarrhea, depression, headache and flu-like symptoms (Cox et al., 2005; Sheyin et al., 2011). Virtually all persons infected with HCV show evidence of inflammation on liver biopsy, however the rate of progression of liver scarring (fibrosis) shows significant variability among individuals. The progression of the disease has been found to be influenced by some factors such as age, gender (males have more rapid disease progression than females), alcohol consumption, HIV co-infection, Hepatitis B vaccine (HBV) co-infection, serum aminotransferase level, viral load, genotype and fatty liver (Ejiofor et al., 2010). Studies have shown that with the introduction of routine screening for Hepatitis B surface antigen (Hbs-Ag) in the serum of blood donors has reduced the incidence of post-transfusion hepatitis B infection (Okafor and Obi, 1979). Apart from hepatitis B, HCV may be seen as cause of complications of blood transfusion. Hence, this research was carried out to determine the seroprevalence of hepatitis C virus infection among the blood donors in Southern part of Kano State, Nigeria.

## MATERIALS AND METHODS

### Study area/population

Blood samples were collected from four blood banks in the general hospitals of Wudil, Gaya, Sumaila and Community/NYSC Comprehensive Health Centre, Takai. Consenting blood donors were recruited for the study when they came to laboratories for blood donation.

### Sample collection and handling

A total of 320 volunteers who consented and completed

questionnaire were sampled. The collection was achieved first by applying a tourniquet, which was tied on the upper arm of the donor, the antecubital region was disinfected with 70% alcohol. 3 ml of whole blood was withdrawn aseptically from the cephalic vein of the antecubital fossa of each donor using a sterile syringe. The tourniquet was then removed and the puncture blocked with sterile dry cotton wool and moderate pressure was applied to stop bleeding. The blood was transferred to plain container and allowed to clot for separation (Chesebrough, 2005).

### Separation and storage of serum

The clotted blood was spun at 3000 rpm for 10 min, the serum separated into a sterile plain container(s) and stored at -20°C before use. The analysis was carried out using third-generation enzyme-linked immunosorbent assay (ELISA) (manufactured by Clinotech Diagnostics and Pharmaceutical Inc, Canada).

### Test procedure

The test kit with the reagents and specimens were allowed to equilibrate to room temperature before the assay was carried out. The micro-titre plates coated with HCV were removed from the sealed bags and used immediately. One hundred microliter of specimen diluent was pipetted into each test well (5 wells were left for controls and blank). One hundred microliters of positive and negative controls was pipetted to duplicate wells while 100 microliters of distilled water was pipetted into the blank well. Five microliters of each sample was added into the assignment well, it was vortex to mix and incubated at 37°C for 30 min. Each well was washed 5 times by filling each well with diluted wash buffer (1:25), then inverting the plate vigorously to get all water out and blocked rim of wells on absorbent paper for a few seconds. One hundred microliter of enzyme conjugate was added to each well except blank well and was mixed gently by swirling the micro-titer plate and then incubated at 37°C again for 30 min. It was washed five times. One hundred microliters of 3,5-tetramethyl benzidine (TMB) solution (Horse Raddish peroxidase, substrate) was added to each well, and then incubated at 37°C for 10 min. Fifty microliters of stop solution was added to each well, which stops the color reaction. The optical density (OD) was read at 450nm with a multiscan system (EIA reader).

### Interpretation of results

All absorbance values, for both the controls and the specimens were subtracted by the value of the blank before interpretation. The presence or absence of antibody specific for HCV was determined by relating the absorbance of the specimens to cut-off value.

Calculation of cut-off value:

Cut – off value =  $P \times 10\% + N$ : In this study;  $P = 1.865$ ,  $N = 0.045$

$$\begin{aligned}\text{Cut – off} &= 1.865 \times 10\% + 0.045 \\ &= 1.865 \times 0.1 + 0.045 \\ &= 0.1865 + 0.045 \\ &= 0.2315 \approx 0.232.\end{aligned}$$

A test is positive if  $S \geq \text{cut-off value}$ . A test is negative if  $S < \text{cut-off value}$ .

Where,  $N$  = Mean absorbance of the negative controls.  $P$  = Mean absorbance of the position controls.  $S$  = Absorbance of the test sample.

All absorbance that are greater or equals to 0.232 were considered

**Table 1.** Overall results of the HCV prevalence in the study area.

Total number of sample	Number of reactive (%)	Number of non-reactive (%)
320	16 (5.0)	304 (95.0)
P-value	0.000**	

Key: \*\* indicates highly significant.

**Table 2.** Prevalence of HCV according to Local Government Area.

LGA	Number of reactive (%)	Number of non-reactive (%)
Wudil	5 (6.3)	75 (93.8)
Gaya	5 (6.3)	75 (93.8)
Sumaila	4 (5.0)	76 (95.0)
Takai	2 (2.5)	78 (97.5)
P-value	0.664 <sup>NS</sup>	

Key: NS indicates not significant.

as reactive while all absorbance that were less than 0.232 were considered non-reactive.

### Statistical analysis

The data generated in this study was analysed for statistical significance using Pearson Chi-Square with the aid of statistical package for social sciences (SPSS) version 15.0, Chicago incorporation 2007.

## RESULTS

Out of 320 blood donors involved in the study 16 (5.0%) were sero-positive to HCV while 304 (95.0%) sero-negative (Table 1). Table 2 depicts the prevalence of HCV according to the LGA. Out of the 80 subjects in each LGA, Gaya and Wudil had the highest prevalence rate of 5(6.3%) each followed by Sumaila 4 (5.0%) and Takai had the least prevalence rate of 2 (2.5%); however this did not reveal statistical significant ( $P > 0.05$ ). Table 3 shows the prevalence of HCV in blood donors in relation to demographical parameters. There were differences across these demographical characteristics; however, all are not statistically significant ( $P > 0.05$ ). The relationship among possible risk factors and HCV infection in Table 4 revealed a significant association in transmission through vertical, receiving injection at time of sickness, history of sexually-transmitted infections (STIs), and alcohol consumption while it indicated a non-significant association in transmission through blood transfusion, history of surgery, history of hepatitis and cigarette smoking. All the donors were found to use new syringe as well as not sharing needle with other people when receiving injection each time as such no disease was recorded in each case.

## DISCUSSION

The data on the epidemiology of hepatitis C virus in Nigeria is limited (Ejiofor et al., 2010). However, it has long been suspected that it may be endemic. In the present study, 320 blood donors were screened and 16(5.0%) were sero-positive and all of them were males. This value (5.0%) is lower compared to the findings of Oni and Harrison (1996) who reported a sero-prevalence rate of 8.0% from a study conducted among male adults and children in Nigeria. Another study conducted by Halim and Ajayi (2000) reported a prevalence of 12.3% which is also higher than that obtained in the present study. Chukwurah et al. (2005) also reported a prevalence of 7.6% among blood donors in South-eastern state of Nigeria. Ayolabi et al. (2006) reported a sero-prevalence rate of HCV among blood donors in Lagos, Nigeria to be (8.4%), which is also higher than the one observed in the present study. On the other hand, Beatriz et al. (2000) reported a lower sero-prevalence rate of HCV (1.2%) in the general population of North-western Tanzania. In addition, the finding in the present study is also higher than values ranging between 0 to 1.4% reported from USA and Europe (Alter et al., 1999). These variations could be due the fact that prevalence of HCV greatly differs according to the geographical location of a population. It was indicated that age groups of 24 to 33 and 34 to 43 years had the highest prevalence rate of 6(1.9%) each (Table 3). These age groups represent the sexually-active group of the population hence, showing probably the mode of transmission of the virus. The prevalence rate in relation to the demographical characteristics (Table 3) did not reveal significant association ( $P > 0.05$ ) in all the cases. This means that the characteristics had no effect on the prevalence of HCV even though the prevalence rates were found to

**Table 3.** Prevalence of HCV according to demographical characteristics of the subjects.

Demographic characteristic	Total number examined (%)	Number of reactive (%)	Number of non-reactive (%)
<b>Sex</b>			
Male	319 (99.7)	16 (5.0)	303 (94.7)
Female	1 (0.3)	0 (0.0)	1 (0.3)
Total	320	16 (5.0)	304 (95)
P-value		0.818 <sup>NS</sup>	
<b>Age group (years)</b>			
14-23	56 (17.5)	1 (0.3)	55 (17.2)
24-33	127 (39.7)	6 (1.9)	121 (37.8)
34-43	91 (28.5)	6 (1.9)	85 (26.6)
44-53	35 (10.9)	3 (0.9)	32 (10.0)
≥54	11 (3.4)	0 (0.0)	11 (3.4)
Total	320	16 (5.0)	304 (95.0)
P-value		0.518 <sup>NS</sup>	
<b>Marital status</b>			
Married	265 (82.8)	14 (4.4)	251 (78.4)
Single	55 (17.2)	2 (0.6)	53 (16.6)
Total	320	16 (5.0)	304 (95.0)
P-value		0.610 <sup>NS</sup>	
<b>Education quality</b>			
None	11 (3.4)	2(0.6)	9 (2.8)
Arabic	113 (35.3)	3(1.0)	110 (34.4)
Primary	82 (25.6)	6(1.9)	76 (23.8)
Secondary	71 (22.2)	3(1.0)	68 (21.3)
Tertiary	43 (13.4)	2(0.6)	41 (12.8)
Total	320	16(5.0)	304 (95.0)
P-value		0.174 <sup>NS</sup>	
<b>Occupation</b>			
Public servant	39 (12.2)	1 (0.3)	38 (11.9)
Unemployed	35 (10.9)	3 (1.0)	32 (10.0)
Self-employed	246 (76.9)	12 (3.8)	234 (73.1)
Total	320	16 (5.0)	304 (95.0)
P-value		0.448 <sup>NS</sup>	
<b>Settlement</b>			
Urban	9(2.8)	0(0.0)	9(2.8)
Semi urban	89(27.8)	3(1.0)	86(26.9)
Rural	222(69.4)	13(4.0)	209(65.3)
Total	320	16(5.0)	304(95.0)
P-value		0.519 <sup>NS</sup>	

Key: NS indicates not significant.

vary across some of the characteristics (Table 3). The most common risk factor of HCV in developing countries like Nigeria is transfusion of unscreened blood or plasma-derived products (Conry-Contelena et al., 1996; Erhabor et al., 2006). In this study, the prevalence rate was related to the history of transfusion among the donors where it was observed that all the cases occurred among the donors that did not receive blood transfusion (Table 4). However, this is at variance with previous reports, where it has been established that HCV is transmitted

through blood transfusion. Among the reasons, which cause this disagreement could be that a donor might have been transfused long ago, for example, at child stage, which he was not aware and then responded that he did not receive blood transfusion ever in his life, or he might have been transfused and yet he refused to disclose it. Vertical transmission was reported by Ejiofor et al. (2010) and Jawetz and Adelberg (2007) to occur in the transmission of HCV and this was confirmed in the present study where the prevalence rate occurred higher

**Table 4.** Prevalence of HCV according to possible risk factors.

Possible risk factor	Total Number (%)	Number of reactive (%)	Number of non-reactive (%)
<b>Blood transfusion</b>			
Yes	5(1.6)	0 (0.0)	5 (1.6)
No	315(98.4)	16 (5.0)	299 (93.4)
Total	320	16 (5.0)	304 (95.0)
P-value		0.605 <sup>NS</sup>	
<b>History of liver disease</b>			
Yes	5 (1.6)	1 (0.3)	3 (0.9)
No	316 (98.7)	15 (4.7)	301 (94.1)
Total	320	16(5.0)	304 (95.0)
P-value		0.065 <sup>NS</sup>	
<b>Surgery before</b>			
Yes	5 (1.6)	1 (0.3)	3 (0.9)
No	316 (98.7)	15 (4.7)	301 (94.1)
Total	320	16(5.0)	304 (95.0)
P-value		0.065 <sup>NS</sup>	
<b>Family history of hepatitis</b>			
Yes	19 (5.9)	9 (2.8)	10 (3.1)
No	301 (94.1)	7 (2.2)	294 (91.9)
Total	320	16(5.0)	304(95.0)
P-value		0.000**	
<b>Medication use</b>			
Injection	218 (68.0)	16 (5.0)	202 (63.1)
Tablet	102 (31.9)	0 (0.0)	102 (31.9)
Total	320	16(5.0)	304 (95.0)
P-value		0.005**	
<b>New syringe or re-usable</b>			
New syringe	320 (100)	16 (5.0)	304 (95.0)
Re-usable	0 (0.0)	0 (0.0)	0 (0.0)
Total	320	16 (5.0)	304 (95.0)
P-value		.a	
<b>Sharing needle</b>			
Yes	0 (0.0)	0 (0.0)	0 (0.0)
No	320 (100)	16 (5.0)	304 (95.0)
Total	320 (100)	16 (5.0)	304 (95.0)
P-value		.a	
<b>Suffered from STI before</b>			
Yes	1 (0.3)	1 (0.3)	0 (0.0)
No	319 (99.7)	15 (4.7)	304 (95.0)
Total	320	16 (5.0)	304 (95.0)
P-value		0.000**	
<b>Alcohol consumption</b>			
Yes	4 (1.3)	3 (0.9)	1 (0.3)

Key: \*\*, indicates highly significant. \*, indicates significant. <sup>NS</sup>, indicates not significant, .a indicates inability of computing statistics because of one variable is constant.

in the donors who had the family history of hepatitis infection and statistical analysis revealed a highly significant association ( $P < 0.01$ ). Ejiofor et al. (2010) and Sheyin et al. (2011) also reported alcohol consumption among factors that affect disease progression and this was identified in the present study where the relationship

between the prevalence rate of HCV and alcohol consumption was found to be statistically significant ( $P < 0.01$ ). However, alcohol consumption was found to be a separate factor that plays a role in the progressive transmission of the disease. There is no statistical significant association between cigarette smoking and

Table 4. contd.

No	316 (98.7)	13 (4.1)	303 (94.7)
Total	320	16 (5.0)	304 (95.0)
P-Value		0.000**	
<b>Cigarette smoking</b>			
Yes	9 (2.8)	0 (0.0)	0 (0.0)
No	311 (97.2)	15 (4.7)	296 (92.5)
Total	320	16 (5.0)	304 (95.0)
P-value		0.394 <sup>NS</sup>	

Key: \*\* indicates highly significant. \* indicates significant. <sup>NS</sup> indicates not significant. .a indicates inability of computing statistics because of one variable is constant.

HCV infections as such had no effect on the prevalence rate of HCV infection as observed in this study.

## Conclusion

This study indicated a significant prevalence rate among blood donors, screening blood donors for HCV infection has not been properly incorporated in some blood banks, of public health facilities as well as private health institutions; there is no statistical association between HCV prevalence and demography as well as some possible risk factors. Vertical transmission of HCV was identified in this study.

## RECOMMENDATIONS

There is need to institute national public health intervention programs that will involve mandatory screening of blood and blood products especially for HCV before transfusion and also to promote health education in HCV infection and its prevalence. Government should try to provide vaccines against HCV as it is done with other deadly diseases like polio, measles, HBV, whooping cough, etc. so as to help the citizens in reducing the spread of the disease.

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