

# **Seroprevalence of Hepatitis C virus infection amongst febrile patients attending selected public and private hospitals in Lagos state, Nigeria**

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## **ABSTRACT**

Hepatitis C virus (HCV) is the leading reason for liver transplantation in the world; patients infected with HCV are at increased risk of cirrhosis and hepatocellular cancer. The study was aimed at evaluating the seroprevalence of Hepatitis C virus infection amongst febrile patients attending selected public and private hospitals in Lagos state, Nigeria. The hospital based cross-sectional study took place between October through December 2019. A total of 89 blood samples were collected from febrile patients after informed consent and self-administered questionnaires were completed. The samples were centrifuged, and screened for anti-HCV antibodies using the Enzyme-linked immunosorbent assay (ELISA) technique. The results of this study were analysed statistically, and out of the 89 participants screened, only 5 (5.61%) were positive for anti-HCV Ab. There was no significant difference ( $P > 0.05$ ) between the number of male and female patients positive and also other demographic characterization (age and temperature).

*Keywords: Hepatitis C virus; febrile; ELISA; blood donors; Lagos*

## 1. INTRODUCTION

Hepatitis C is a major public health problem that must be controlled and possibly eradicated. Hepatitis C virus (HCV) was first documented as a cause of transfusion associated with acute and chronic hepatitis in 1989 [1] and plays a key role as a cause of chronic liver injury with potential for neoplastic deterioration. It is mostly transmitted by parenteral route; although with lower effectiveness, it may also be transmitted by sexual intercourse as well as from mother to child. HCV infection is often asymptomatic, making it very hard to detect at an early stage. This is the major reason why early treatment is difficult, therefore, hepatitis C is often referred to as a "silent disease". In many infected people, the virus infection does not resolve naturally. The Neutralizing antibodies appear to be manufactured during a natural infection; yet, the virus transforms to escape surveillance. As Hepatitis C virus infection is transmitted with high efficacy via blood to blood contact, the prevalence of HCV within different countries, regions and populations is closely related to the incidence of blood borne (mainly intravenous drug use) disease. HCV infection is an important public health problem today because it is an emerging epidemic disease which infects nearly 3% of the population worldwide and has emerged as a major causative agent of liver disease, resulting in acute and chronic infections that can lead to fibrosis, cirrhosis and hepatocellular carcinoma. It is estimated that more than 170 million individuals are infected with HCV worldwide, most of them chronically [2].

HCV is accountable for about 350,000 deaths annually; among western countries, Southern Europe and particularly Italy is among the most affected areas [3]. Most people (80%) with acute HCV infection are asymptomatic. If symptoms occur, they may include abdominal pain, loss of appetite, nausea, fatigue, dark urine, and jaundice. Of those who develop chronic HCV infection, the most common symptom is fatigue [4]. Severe liver disease develops in approximately 10%–20% of chronically infected people, but progression to end-stage liver disease is slow and typically does not occur until  $\geq 20$  years after infection. This development is often clinically silent until late in the course of disease, and in the absence of HCV testing, most people are unaware of their infection.

Africa has the highest WHO estimated regional prevalence (5.3%) with Egypt having the highest prevalence (17.5%) of HCV in the world; Also, many HIV-positive persons in sub-Saharan Africa are co-infected, a systematic review and Meta-analysis, showed anti-HCV prevalence rates of 7% among HIV infected individuals. The epidemiology of HCV infection in Nigeria is not well understood although the prevalence among high risk groups was given as 12.3% [5]. Majority of research on prevalence of hepatitis C in Nigeria have focused on serological characterization of selected population groups, e.g. patients with diabetes mellitus, prison inmates, HIV-infected persons, blood donors, patients with chronic renal failure and those with sickle cell anemia, for whom risk for HCV infection in urban areas of Nigeria is variable. Previous studies established a broad HCV-seroprevalence rate, ranging from 1.9% among pregnant women in Benin City to 14.5% among apparently healthy individuals with a family history of diabetes in Plateau State [6] or among HIV-positive patients in Lagos [6]. HCV infections have been shown to play a significant role in the aetiology of chronic liver disease and Hepatocellular carcinoma (HCC) in Nigeria. However, a study of adolescent and adult patients with sickle cell anemia (SCA) in Benin by [7] showed 20% prevalence rate. Another study in Ibadan conducted among doctors recorded a seroprevalence of 11% [8]. It was also noted that HCV infections were found more in lower socio-economic class than other social classes. In Nigeria, 18.7% of liver cancer patients carry markers of HCV and it is said that the results of seroprevalence studies of HCV in Nigeria vary depending on the study population and the geographical setting having higher rates along the eastern borders and some in Northern regions [9].

The challenge arises that there is dearth of information on burden and circulation of HCV in Nigeria (Lagos State). The risk factors for HCV transmission in Nigeria have not been properly characterized and the seroprevalence of HCV infection amongst unhealthy, immunocompromised patients and carriers in Lagos is unknown. Therefore, this study was carried out to evaluate the Seroprevalence of Hepatitis C virus infection amongst febrile patients attending selected public and private hospitals in Lagos state.

## 2. MATERIAL AND METHODS

## **2.1 Study Area**

Alimosho Local Government Area is home to the General Hospital Alimosho, Igando. It is the largest local government in Lagos state with coordinates  $6^{\circ} 36'38''N/3^{\circ} 17'45''E$ . It has a total population of about 1,362,077, and land area of  $185 \text{ km}^2$  with average density of 713 persons per square kilometer approximately. The study area Igando, as illustrated in Figure 3.2, is bounded in the North and West by River Owo and Ifakoljaiye, Agege respectively, and the East by Ikeja Local Government Area while it is bounded in the South by Oshodi/Isolo, Amuwo-odofin and Ojo local Government Areas of Lagos State[10]

Lagos state University Health Centre is the primary health care clinic available to the staffs and students of Lagos state University at Ojo. Ojo Local Government Area has a total population of about 598,071 comprising 310,100 males and 287,971 females (NPC. 2006) and land area of 375 sq. km. It occupies the south western part of the Lagos metropolis. It is located between latitude  $6^{\circ} 22'N$  and  $6^{\circ} 32'N$  and on longitude  $3^{\circ} 4'E$  and  $3^{\circ} 20'E$ . The local Government Area is bounded by six other local government areas and is among the seven Local Government Areas occupying the coastal plain of Lagos metropolis. The predominant land uses in this area are residential and commercial land use. Commercial land use includes financial institution and market[11].

Agbara is located at the west of Ologe lagoon and North of Badagry creek, in Lagos state southwest Nigeria, on longitude of  $2^{\circ} 42'1''E$  and  $3^{\circ} 23'1''E$ , and latitude of  $6^{\circ} 23'1''N$  and  $6^{\circ} 28'1''N$ . It is an industrial area with various kinds of industries including Pharma-Deko and Unilever and Ibijola medical center is also situated there[12]

## **2.2 Study Centre**

This was a hospital based cross-sectional study that covered three local government areas in Lagos State. The hospital samples were collected from are General Hospital Alimosho, Igando (AGH), Lagos State University Health Center (LSUHC), Ibijola Medical Center Agbara (IMC). Eighty-nine (89) blood samples were collected from patients in the phlebotomy section of the hospitals mentioned above (20 samples from AGH, 35 samples from IMC and 34 samples from LSUHC). Ethical clearance with reference number (LSHSC/2222/VOL. VB/66) was obtained from the Lagos State Health Service Commission. A structured questionnaire was designed and administered, in order to obtain demographic information, characteristics, personal (such as age, sex).

## **2.3 Study population**

The collection of samples was based on adult and Children febrile patients/clients attending Alimosho General Hospital, Lagos State University Health center, Ibijola Medical Center Lagos State.

## **2.4 Sample collection and Transportation**

Blood samples were collected from each febrile patient from the month of October 2019. All study subjects were between 18-50 years of age who must have had fever at the point of collection or symptoms related over the last six months i.e. patients whose temperature was above  $37.5^{\circ}C$ . A total of 89 blood samples were collected by venipuncture using sterile 5 ml syringe /10ml EDTA Vacutainer and transported in cold box to the Centre of Human Virology and Genomics, Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State.

The samples were centrifuged (Eppendorf) at 3500 rpm for 10 minutes and the Plasma (supernatant) aliquot was transferred into cryogenic vials and stored in the refrigerator ( $-4^{\circ}C$ ) until ready for analysis. All necessary precautions were taken to guide against interference that could negatively affect the result

## **2.5 Serological Technique**

### **2.5.1 Principle of the test**

The micro plates are coated with HCV-specific antigens derived from "core" and "ns" region encoding from conservative and immune dominant antigenic determinants (Core peptide, recombinant NS3, NS4 and NS5 peptides). The solid phase is first treated with the diluted sample, in the 2nd incubation bound HCV antibodies, IgG and IgM as well, are detected by the addition of polyclonal specific anti HCV antibodies, labeled with Horse Radish Peroxidase (HRP). The enzyme captured on the solid phase, acting on the substrate/ chromogen mixture, generates an optical signal that is proportional to the amount of anti-HCV antibodies present in the sample. A cut-off value let optical densities be interpreted into HCV antibody negative and positive results.

### **2.5.2 HCV Detection using ELISA Kit**

The HCV antibodies were detected using qualitative RecombiLISA HCV ELISA kit manufactured by DIA.PRO Diagnostics Bioprobes. All reagents and controls (positive and negative) were brought to room temperature. The concentrated washing buffer was diluted 30-fold with distilled water. The HCV-Ab test kit used was an Enzyme Immunoassay kit used for the determination of anti-Hepatitis C Virus antibody in human serum and plasma and it is an in-vitro diagnostic test. The procedure used is an Enzyme Linked Immunosorbent Assay (ELISA).

### 2.5.3 Assay Procedure

The first well was left empty for the operation of blanking. Next 200ul of negative control was dispensed in triplicates, 200ul calibrator was placed in duplicate and 200ul of positive control is dispensed in a single well. Then 200ul of Sample Diluent (DILSPE) was added to all the sample wells except the calibrator and controls which were already pre-diluted, 10ul of sample was dispensed in each properly identified well and mixed gently. 50ul of Assay Diluent (DILAS) was also dispensed into all the controls/calibrator and sample wells. The micro plate was incubated for 45min at 37°C and the plate was washed with an automatic washer 6 times. 100ul of the Enzyme Conjugate was pipetted into each well, except the 1st blanking well and was then covered with a sealer. The micro plate was incubated for 45min at 37°C, and after incubation the micro wells were washed 6 times using an automatic washer. 100ul Chromogen/Substrate mixture was pipetted into each well and the blank included. Then incubated the micro plate at room temperature (18-24°C) for 15mins. 100ul of sulphuric Acid was pipetted into the wells using the same sequence as the substrate to stop the enzymatic reaction. Addition of acid will turn the positive control and positive samples from blue to yellow/brown. Finally, the optical Density (OD) was read at 450nm wavelengths within 15minutes of stopping the reaction using GF-M3000 micro plate reader and results were read according to the manufacturer's manual.

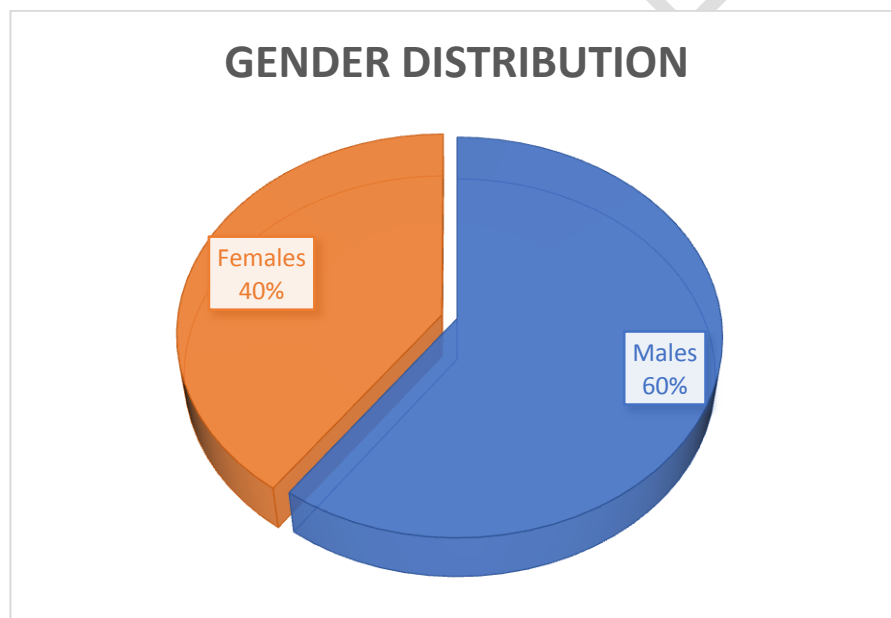
## RESULTS

A total of 89 plasma samples were tested for Hepatitis C virus antibodies in addition with the reagents used (controls and calibrations) on the micro plate and an Enzyme Linked Immunosorbent Assay (ELISA) reader was implored for the reading of the optical density. Among the 89 blood samples collected from febrile patients in the three selected hospitals, 5(5.62%) were positive for HCV antibody (Table 1). The demographic characteristics of the patients showed age range of 18 - 26, the overall mean age of the 89 patient's blood samples collected across the 3 selected public and private hospital was 25years (age range 18 – 26). The highest proportion of HCV antibody seropositive result was recorded most among males whose age are below 30. The gender distribution of the positive patients was shown in figure 2

**Table 1: Prevalence rate of Hepatitis C virus antibodies in each study area**

Hospital	Frequency (n=89)	No of positive result	No of negative result	Percentage of positive results (%)
Ibijola medical center	35	3	32	3.37
Alimosho general hospital	34	0	34	0
Lagos state university health	20	2	18	2.24

center				
Total	89	5	84	5.61



**Figure 2: Gender distribution of HCV antibody positive patients from Ibijola medical center and Lagos State University Health Center.**

## DISCUSSIONS

HCV is widespread in the world and in Lagos state. It is among the important hepatotropic viruses because of liver tropism which leads to cirrhosis or hepatocellular carcinoma. This has become an issue of public health concern. The RecombiLISA HCV Ab ELISA is able to qualitatively detect antibodies (both IgG AND IgM) to Hepatitis C virus in human serum or plasma and has become an important tool to identify individuals with early infection so as to administer early antiviral therapy.

The prevalence of HCV Ab among the febrile patients in this study was 5.61%. Though no work has been done pertaining to febrile patients in Lagos or Nigeria, the prevalence is greater than zero (0%) recorded by Enitan *et al.*, in 2019[13] among undergraduate students of Babcock University Ogun State. Work done by Muhibi *et al.*, [14]. and Alquatani *et al.*, [15], reported zero (0%) prevalence of anti-HCV antibody among undergraduate students of Achievers University, Owo in south-west Nigeria, as well as among Health

Students in the Najran region of South-Western Saudi Arabia, respectively. The outcome of this study however is also greater than the work of Hebo *et al.*, [16] who reported a prevalence of 0.42% among Health Workers of University Medical Center, Southwest Ethiopia, as well as that of Jemilohun *et al.*, [17] who reported a prevalence rate of 0.40% among undergraduate Student of Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo State, south-west Nigeria.

The results of this study is lower than the work of Udeze *et al.*, [18] who reported a prevalence rate of 8.0% among first year Students of University of Ilorin, Kwara State, Nigeria. In a recent study by Tula *et al.*, [19] a much higher prevalence rate of 11.5% was recorded among Students of Federal Polytechnic Mubi, Adamawa; majority of whom had history of blood transfusion, medical surgery and circumcision

Gender was not found to be associated with the viral prevalence although the infection was higher among males than females ( $P > 0.05$ ), but this may be connected to the fact that some men involve in homo sexualism which is a high risk factor for the transmission of HCV as Risky sexual behaviors such as fisting and unprotected intercourse can be mucosally traumatic and may be associated with bleeding [20]. Whether bleeding is necessary for HCV transmission is still debatable though some studies have identified HCV in seminal and rectal fluids of HIV infected men and providing evidence that these fluids can mediate HCV transmission [21] or due to having numerous sexual partners due to promiscuous lifestyle.

The age stratification in this study shows no statistical significance with age in HCV prevalence. HCV Ab was detected more among patients that were between the age 18 – 26 years This might be suggestive that those in such age are more active and likely to engage in unprotected sex and are also not aware of immunization.

Temperature was not found to be associated with the prevalence despite the fact that fever is a symptom of acute stage HCV but this might be that some patients had other serious infection such as typhoid or malaria at the time of blood collection.

## **CONCLUSION**

After all test and serological analyses carried out the research indicate 5 positive HCV Ab among tested blood patients, which gave a seroprevalence rate of 5.61% in the study area and this indicated that HCV is endemic in our environment though with a low prevalence rate, it should be taken into account by doctors and nurses in hospitals when treating febrile patients as drug interactions could potentially increase morbidity in this population. The information provided by the study can be used to provide baseline data that can contribute to knowledge on the magnitude of the disease, stimulate further research on the disease and inform policy on risk assessment. Our study has some limitations. First, in the number of people we involved, as more test subjects would yield more reliable results. Second, it would have been useful to confirm the presence of the hepatitis viruses with a nucleic acid-based technique such as Polymerase Chain Reaction, Nonetheless, our results are consistent with other studies, and are relevant for improving the care of HIV/AIDS patients. Compulsory public awareness campaigns against HCV infection and prevention programs should be intensified to eradicate future outbreak cases of HCV in the country.

## **CONSENT**

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report.

## **ETHICAL APPROVAL**

An ethical approval with reference number (LSHSC/2222/VOL. VB/66) was sought from the Lagos State Health Service Commission (LSHSC). An approval was also given from the Nigerian Institute of Medical Research (NIMR) an institutional review board to carry out the laboratory work in their Human genomics and molecular laboratory. An approval was also sought from the respective Chief Medical Director (CMD) of the Hospitals. The following information was given to each participant to ensure that they make an informed choice; a complete description of the aims of the study, infectious agent that was being screened, details of sample collection procedures, potential benefits and risks of their participation in the study and assurance of confidentiality of any information given as well as of the test results, all these

were explained to the subject in English language and/or, their native languages and consent were sought through the signing of informed consent form. Dignity of the study participants was upheld throughout the study.

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