Original Research Article

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

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. The hospital based cross-sectional study took place between October – 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 HCV Ab using the Enzyme-linked immunosorbent assay (ELISA) technique. Out of the 89 participants screened only 5(5.61%) were positive for 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). The outcome of this study showed that a low prevalence rate of HCV exists among patients attending the selected hospitals in Lagos State. Therefore, the on-going public health campaign programme against Hepatitis C should be sustained.

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

1. INTRODUCTION

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 ≥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]. Most researchs 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 anaemia, 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 anaemia (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].

Hepatitis C is a major public health problem that must be controlled and possibly eradicated. 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 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 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).

2.2 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. 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 37.5^0 c.

2.3 Ethical Approval and Permission

An ethical approval 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.

2.4 Sample collection and Transportation

Samples were collected from the months of October – December 2019. 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.

2.5 Sample storage

The samples were respectively centrifuged (Eppendorf) at 3500 rpm for 10 minutes and the Plasma (supernatant) aliquot was transferred into cryogenic vials and stored in the refrigerator (-4^0c) until ready for test use. All necessary precautions were taken to guide against interference that could negatively affect the result.

2.6 Materials and reagents

2.6.1 Reagents

- Controls (positive and negative)
- Calibrations

2.6.2 Materials

- Cryovials
- K-tips
- Eliza reader
- Micro-pipette

2.7 Variables

Dependent variables – Hepatitis C virus infection,
Independent variables- Age, sex (gender defined as male/female)

2.8 Serological Technique

2.8.1 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 higG&M 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.

2.8.2 Procedures for detection

The HCV antibodies were detected using qualitative RecombiLISA HCV ELISA kit manufactured by DIA.PRO Diagnostics Bioprobes Srl. 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).

2.8.3 Assay Procedure

1. The first well was left empty for the operation of blanking 200ul of negative control is dispensed in triplicate, 200ul calibrator is placed in duplicate and 200ul of positive control is dispensed in a single well
2. 200ul of Sample Diluent (DILSPE) was then added to all the sample wells except the calibrator and controls which were already pre-diluted, the 10ul of sample was dispensed in each properly identified well and mixed gently
3. 50ul of Assay Diluent (DILAS) was dispensed into all the controls/calibrator and sample wells
4. The micro plate was incubated for 45min at 37^{o}c
5. The plate was washed with an automatic washer 6 times
6. 100ul Enzyme Conjugate was pipetted into each well, except the 1st blanking well and then was covered with a sealer
7. The micro plate was incubated for 45min at 37^{0}c
8. After incubation the micro wells were washed 6 times using an automatic washer
9. 100ul Chromogen/Substrate mixture was pipetted into each well and the blank included. Then incubated the micro plate at room temperature (18-240c) for 15mins
10. 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

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.

2.16 Interpretation of ELISA Results

(a) Set up of the cut-off value
The cut-off value (Co) = 0.350+ NC
NC: mean OD of the negative control
Nc of the test= 0.085
Cut-off value (Co) = 0.350 + 0.085
(Co) = 0.435

(b) Calculation of OD specimen ratio
Specimen OD ratio was calculated by dividing specimen OD by the cut-off value as follows:
Specimen OD ratio = specimen OD/ Cut-off value

(c) Chart1 Interpretation of the results

S/Co	**Interpretation**
≤0.085	Negative
0.085 – 0.435	Equivocal

> 0.435	positive

- A negative result indicates that the patient has not been infected by HCV or that the blood unit may be transfused.
- Any patient showing equivocal result should be tested again on a second sample taken 1-2 weeks later from the patient and examined. The blood unit should not be transfused
- A positive result is indicative of HCV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

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, Figure 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 male whose age is below 30. The Optical density values and the gender distribution of the positive patients were shown in table 2 and figure 2 respectively.

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 center	20	2	18	2.24
Total	89	5	84	5.61

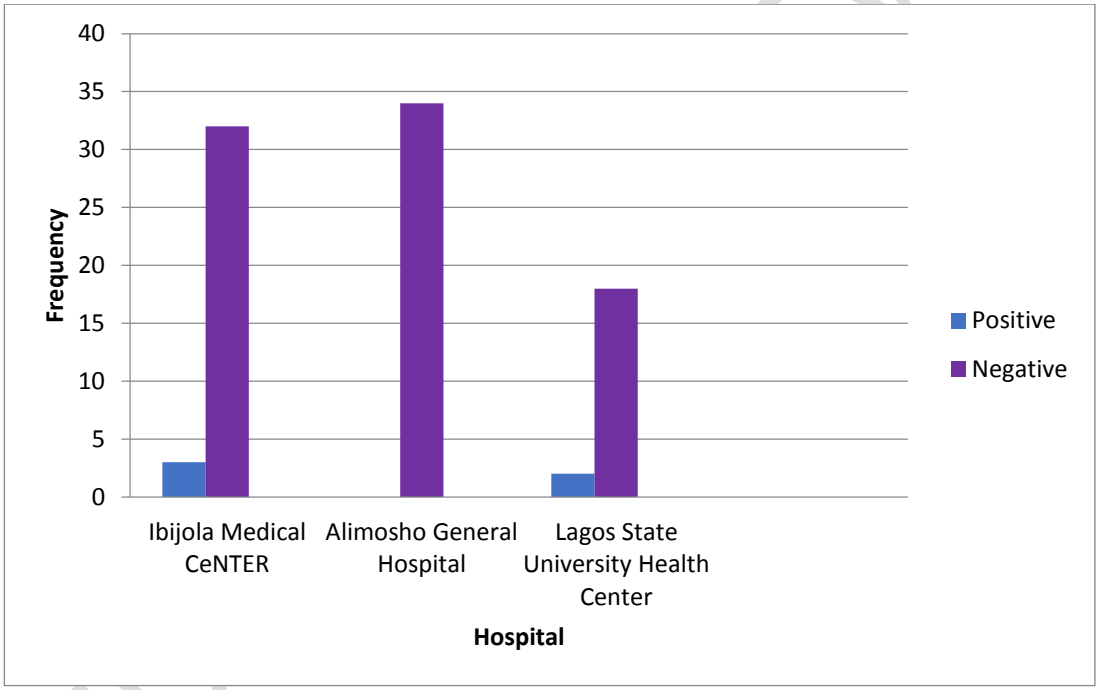

Figure 1: Prevalence rate of HCV antibody in Alimosho general hospital, Ibijola medical center, Lagos state university health center

Table 2: Optical density value of HCV antibody positive patient from Ibijola medical center and Lagos State University Health Center

Sample ID	Hospital	Age	Temperature	Optical density	S/CO
IMC 001	Ibijola Medical Center	18 – 26	36.4	0.206	0.474
IMC 037	Ibijola Medical Center	18 – 26	35.7	0.304	

IMC 022	Ibijola Medical Center	18 – 26	36.0	0.198	0.455
LASU 049	Lagos State University Health Center	18 – 26	35.9	0.221	0.508
LASU 042	Lagos State University Health Center	18 – 26	37.2	0.248	0.570

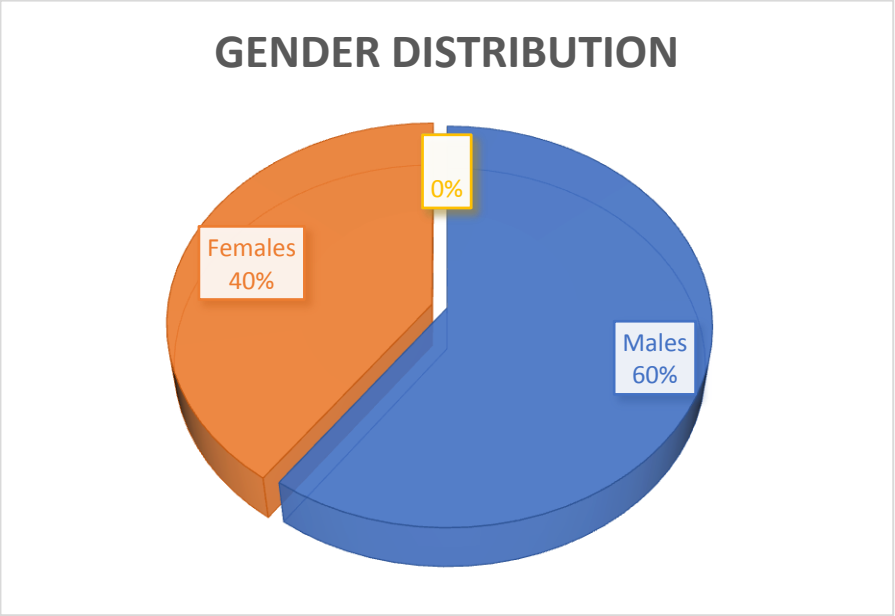

Figure 2: Gender distribution of HCV antibody positive patients from Ibijola medical center and Lagos State University Health Center.

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 cihorrsis 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.

According to the cut off value derived, specimen Optical Density(OD) divided by the cut off value gives the mark for positive and negative result, cut off value that exceeds 0.435 is referred to as the positive result while result lower than the negative cut off value of 0.085 is referred to as the negative result which means no HCV Ab. Results found in between 0.085 – 0.435 are referred to as the equivocal result which means the test have to be carried out again after 1 – 2 weeks and examined for the virus.

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 but the prevalence is greater than zero (0%)

prevalence rate recorded by Enitan *et al.,* in 2019 [10]among undergraduate students of Babcock University Ogun State, work done by Muhibi *et al.,*[11]. and Alquatani *et al.,*[12], who both 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 also greater than the work of Hebo *et al.,* [13] who reported a prevalence of 0.42% among Health Workers of University Medical Center, Southwest Ethiopia, as well as that of Jemilohun *et al.,*[14] who reported a prevalence rate of 0.40% among undergraduate Student of Ladoke Akintola University of Technology (LAUTECH), Ogbomosho, Oyo State, south-west Nigeria.

The study is lower than the work of Udeze *et al.,* [15] 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.,*[16] 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 [17]. 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 [18]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 while anti-HCV was more among those. 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

The study was set out to determine the seroprevalence of HCV infection in Lagos State with some selected hospitals which has been listed above. 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. 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. 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 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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