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**Original Article**

# Molecular Detection of Hepatitis C Virus amongst Patients in Five Selected Hospitals in Niger State, Nigeria

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## ABSTRACT

Hepatitis C Virus (HCV) is a major public health problem in developing and developed countries worldwide. It is responsible for liver diseases and hepatocellular carcinoma in chronically-infected patients. This study therefore aimed to identify the strain of HCV among HCV seropositive subjects in Niger State. A total of 44 HCV seropositive blood samples which consisted of 27 males and 17 females were analyzed (after Viral RNA extraction) for the presence of HCV-RNA by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Nine (20.5%) of the samples were positive for HCV RNA. HCV-RNA positive samples were genotyped by direct sequencing at 5'UTR region genomes; sequences were aligned on MEGA 6.0 and confirmed by phylogenetic analysis. HCV genotype 1b was the only one distributed among the participants. The findings are relevant as predictors for using antiviral therapy in this population because the response to treatment varies according to the genotype.

***Keywords***: *Blood samples, Genotype, Hepatitis C, Liver, Phylogenetic analysis, RT-PCR.*

## INTRODUCTION

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Hepatitis C virus (HCV) is estimated to infect 170 million people worldwide. It was first identified in 1989and is the main cause of chronic liver diseases including cirrhosis (60–85 %) and hepatocellular carcinoma,[1] with a yearly mortality rate of 3.5-5.5 million resulting from complications of end stage liver diseases.[2] HCV is an enveloped virus that belongs to the genus *Hepaci virus* in the family *Flavi viridae.* The genome is composed of 9.5 kilobases of single-stranded, positive-sense RNA that code for at least 10 viral proteins. The polyprotein consists of structural (C, E1, and E2) and non-structural (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins which are flanked by 5′ and 3′ untranslated regions (UTR).[3, 4]

HCV isolates are classified into six major genotypes (genotypes 1–6) and recently a novel genotype 7 has been described as well .[5] HCV genotypes can be divided further into subtypes of different HCV genotypes which include 1a, 1b and 1c in genotype 1; 2a, 2b and 2c in genotype 2; 3a, 3b and 3k in genotype 3; 4a in genotype 4; 5a in genotype 5 and 6a, 6b and 6d in genotype.[5] Genotypes 1, 2, and 3 have a worldwide distribution, while genotypes 4, 5 and 6 are mainly encountered in Egypt, Middle East and Central Africa, Southern Africa and Asia, respectively.[6,7] Divergent strains of genotypes 1 and 2 have been shown to be endemic in West African countries including Burkina Faso, Ghana, Guinea Bissau, Benin Republic and Nigeria.[8, 9] Genotypes 1a, 1b, 2b, 2c, 5a and 4 have been identified and reported in Nigeria.[10,11,12] Genotype identification is clinically significant to predict response to antiviral therapy, because some genotypes are more resistant to treatment than others; it is now routinely done before treatment and serves as a guideline to determine the period of the therapy.[13, 14] It is also useful for molecular epidemiology studies. The

world distribution of HCV genotypes and subtypes differs significantly from a region to another.[15] The aim of this study is to determine the hepatitis C virus strains circulating in Niger State as information obtained will be useful for epidemiological pattern and beneficial for therapeutics of HCV infection in this area and Nigeria at large.

## MATERIALS AND METHODS

### Study Population

This study was carried out in Niger State, Nigeria, amongst 44 HCV seropositive patients using ELISA technique in five selected hospitals [4 from General Hospital Minna (GHM), 14 from General Hospital Kontagora (GHK), 8 from Umaru Sanda Ndayako Hospital Bida (USNHB), 15 from General Hospital Wushishi (GHW) and 2 from General Hospital Suleja (GHS)]. Sera of forty-four participants were analyzed, of which 27 males and 17 females, with age ranging from 1 to 60 years and above. Ethical approval for this study was obtained from each hospital Ethical Review Committee and Niger State Ministry of Health before the study commenced.

### Study Design

The study was hospital-based and covered the three senatorial zones in Niger State. The samples were collected from General Hospital Minna, General Hospital Kontagora, Umaru Sanda Ndayako Hospital Bida, General Hospital Wushishi and General Hospital Suleja. Five hundred and sixty-five (565) blood samples were collected from patients in the phlebotomy section of the hospitals, screened using ELISA method which resulted in 44 seropositive samples. A structured questionnaire and consent forms were designed and administered, in order to obtain demographic information and behavioural risk factors. The laboratory methodology employed was Enzyme-linked immunosorbent assay (ELISA), Extraction, Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR), DNA sequencing and Phylogenetic analysis. Ethical approval was obtained from Research and Ethics Committee of the five hospitals mentioned above.

As mentioned above, blood samples were collected from the Phlebotomy section of the hospitals by trained medical personnel. The blood samples were aseptically collected through the veins using sterile 5 ml syringe and needle, and introduced into sterile test tube and allowed to cloth. The blood samples were respectively centrifuged at 2000 rpm for 2 minutes and the sera were taken and stored in cryovials in the refrigerator ($4^0$C for 7 days or $-20^0$C for longer period) for further analysis. The sera were transported on frozen ice pack to a DNA Laboratory in Kaduna for screening and molecular analysis. All necessary precautions were taken to guide against interference that could negatively affect the result namely; haemolyzed blood samples were rejected, use of sterile materials such as syringe and needle and storage of samples at the required temperature were observed. The services of other Medical Laboratory Scientists were employed during analysis of the samples.

### Sample Size

The sample size was calculated using the equation below;

$$n = \frac{Z^2 P (1-P)}{d^2}$$

Where:

$n$ = required sample size,

Z = level of confidence at 95% (standard value of 1.96)

P = Known prevalence of the disease, 7.7% (which is equal to 0.08) among blood donors in Kaduna.[12]

d = precision or margin of error at 5% (standard value of 0.05)

Therefore, the minimum sample size, $n = \frac{Z^2 P (1-P)}{d^2}$

$$n = \frac{1.962 \times 0.08(1-0.08)}{0.05^2}$$

$$n = \frac{3.8416 \times 0.08 (0.92)}{0.0025}$$

$$n = \frac{0.28274176}{0.0025}$$

$$n = 113.096704$$

$$n = 113.1$$

$$n = 113$$

Therefore, 113 samples was the minimum sample size for the research. The minimum sample size was collected in each hospital given a total of 565 blood samples collected in the ratio 1: 1: 1 respectively. This is to increase the power of the study.

**RNA extraction using Bionner extraction machine**

**Reagents**: The Exiprep™ RNA Kit contains Buffer Cartridges 1& 2, Elution Tubes, Sample Loading Tubes, Sample Loading Tips and Waste Tray.

## Principle of the test

Viral RNA is extracted from clinical samples by using a lysis buffer (Buffer Cartridge 2) to disrupt viral structure. The binding buffer contains guanidine thiocyanate, which act as a chaotropic agent. The binding buffer disassociates water molecules from nucleic acids and silica magnetic beads. This induces negative charge to nucleic acid and positive to silica magnetic beads. As a result, exposed viral RNA binds to the surface of beads. Then magnetic field is used to genomic DNA from impurities. The washing buffer (Buffer Cartridge 1) rinses any impurities that may exist. The elution buffer dissolves pure viral RNA from the beads.

## Procedure

The serum was brought to room temperature and allowed to thaw. The serum was vortexed to homogenize. Cartridges 1 & 2 were punctured using a hole puncher. The sample loading tubes were labeled accordingly with numbers 1-16. After that the elution tubes were labeled from 1-16 accordingly and covered with its appropriate cover. 400µl of the samples were pipette into the loading tubes. The items were then arranged accordingly on the extraction machine as follows; the Buffer Cartridge 2, Buffer cartridge 1, Sample Tube Rack, Waste tray, Elution tube Rack and the Disposable Tip Rack in that order. The machine was then set up using the appropriate code (513) for RNA extraction. After that, the start button was pressed for the extraction to commence which last for 1 hour 32 minutes. At the end of the extraction, RNA were removed and placed on ice immediately for reverse transcriptase- polymerase chain reaction or stored at -20$^0$c in the refrigerator.

## Reverse Transcriptase- Polymerase Chain Reaction

## Reverse Transcriptase

Primers HCV – 209 (forward) and HCV – 939 (reverse) were used for the first round reverse transcription. 12µl of deionised water was added to all tubes and this was followed by the addition of 7µl extracted RNA to the respective tubes. 7µl of positive control (HCV positive sample) was added to the positive control tube and 0.5µl each of forward and reverse primers was added to make 20µl final volume. To prevent evaporation one drop of mineral oil was added to the tubes. The tubes were then placed in an already set thermo cycler (PTC – 100TM MJ – Research, INC, Peltier ). Reverse transcription was activated at 42$^0$C for 1hr to obtain a complementary DNA with Moloney Murine Leukemia Virus as enzyme reverse transcriptase enzyme and deactivated at 94$^0$C for 5 min.

**Table 1:** First round cycling conditions for HCV Genome amplification

| Step | Temperature ($^0$C) | Time | Cycle |
|---|---|---|---|
| Pre-denaturation | 94 | 5 min | |
| Denaturation | 94 | 30 sec | |
| Annealing | 53 | 30 sec | 30 cycles |
| Extension | 72 | 30 sec | |
| Final extension | 73 | 2 min | |
| Hold | 4 | Infinity | |

## Polymerase Chain Reaction

Primers KY-80 (forward) and KY- 78 (reverse) were used for the second round polymerase chain reaction. 16µl of deionised water was added to each PCR tube and this was followed by 1µl of each primer added to the tubes including the negative control (Premix) tube. 2µl of first round products (cDNA) was then added to the respective tubes making a final volume of 20µl. The premix used contained 1X Tri- actate- EDTA (TAE) buffer, MgCl, dNTPs (GTP, CTP, ATP and TTP) and Tag polymerase. The PCR tubes were centrifuged at 11,000rpm for 30sec and placed in the thermo cycler for amplification. The primers that were used for the RT-PCR is shown in Table 12.2 (i.e. Primers with sequences obtained from conserved untranslated region of the HCV genome-5'UTR). HCV -939 (sense) and HCV- 209 (antisense) were used for reverse transcription while KY-80 (forward) and KY-78 (reverse) were used for PCR.

**Table 2:** Second round cycling conditions for HCV Genome amplification

| Step | Temperature ($^0$C) | Time | Cycle |
|---|---|---|---|
| Pre-denaturation | 94 | 5 min | |
| Denaturation | 94 | 30 sec | |
| Annealing | 60 | 30 sec | 35 cycles |
| Extension | 72 | 30 sec | |
| Final extension | 73 | 5 min | |
| Hold | 4 | Infinity | |

**Table 3:** Primer sequence used for RT-PCR

| Prime | Sequence | Basepair | References |
|---|---|---|---|
| HCV-93 | 5′-CTGTGAGGAACTACTGTCTT-3′ | 240 | Yang *et al* 2014 |
| HCV-20 | 5′ATACTCGAGGTGCACGGTGCACGGT CTACGAGACT-3′ | | |
| KY-80 | 5′-GCAGAAAGCGTCTAGCCATGGCGT-3′ | 244 | Young *et al.*,1993 |
| KY-78 | 5′-CTCGCAAGCACCCTATCAGGCAGT-3′ | | |

## Agarose Gel Electrophoresis

A 1.5g of agarose (QD LE Agarose, Green BioResearch, USA) was weighed with a weighing balance and poured in a conical flask and mixed with 100ml of Tri Acetate EDTA ( 1x TAE) buffer. The conical flask was then heated in a microwave for two minutes to dissolve the powder completely and the gel allowed cooling to a temperature tolerated by the cheek. 12µl of ethidium bromide stain (intercalating agent) was added to the gel and rocked gently to mix properly. After that, the mixture was poured into a gel casting glass containing two combs placed at distance apart and allowed to solidify for 50 minutes. On solidification, the combs were gently removed and the cast placed in the electrophoresis tank and1x TAE buffer was poured to cover the cast. 7µl PCR products were mixed with 1µl loading dye (bromophenol blue) and dispensed into the various wells in the gel. 8µl of the ladder and negative control were also added into their respective wells after which electromotive force was applied to the gel and run at 120volts for 35min. The gel was removed from the gel box, excess buffer from the surface of the gel was drained off and the gel tray placed on paper towels to absorb any extra running buffer. The gel was removed from the tray, exposed to UV light and pictures taken with a gel documentation system (Gel Doc 2000, BIORAD, USA) to determine the 244bp of the untranslated conserved region.

## Large Reaction

The procedures are the same with second round PCR which is followed by gel electrophoresis. The marker and PCR products were dispensed into five PCR tubes instead of one to have more concentration and placed in a thermo cycler to undergo second round cycling conditions. Here, the gel was prepared using a bigger cast and combs which gave bigger wells. The marker and PCR products (all five tubes) were placed in the respective wells and gel exposed to electromotive force using appropriate volts and time. The gel was exposed to UV light and pictures taken with a gel documentation system as previously done.

## DNA Clean Up

The DNA fragments were excised from the agarose gel using a clean scalpel, (all excess agarose was removed) placed in a 1.5ml microtube and weighed. Sample was incubated using heating block (BioBlock Scientific, Thermolyne Corporation, Iowa, USA) for 10 min at 50°C after adding 3 gel volume buffer GB. The sample was mixed (Vortex mixer- Vision Scientific Co. Ltd, Korea) briefly every 2 min until the gel was completely dissolved. 300µl of Isopropanol was added into each tube and mixed.750µl of the mixture was transferred to a DNA mini column with a collection tube and centrifuged at 11,000rpm for 1 min. The flow through was discarded into a container and columns put back to the collection tubes. This step was repeated until the mixture is completely removed. 750µl of the DNA wash buffer was added to the columns and centrifuged at 11,000rpm for 1 min and allowed to stand for 5 min to enhances membrane clean up to get good DNA. The columns were then spun at 11,000rpm for 1 min and the flow through discarded. 800µl of wash buffer was then added to the columns and centrifuged at 11,000rpm for 1 min and the flow through discarded. The empty columns were centrifuged at 14,000rpm for 2 min to remove residual ethanol for optimal elution. 25µl of deionized water was added to all the columns placed in a clean 1.5ml micro-centrifuge and placed inside block heat at $50^0$C for 1 min ( the deionized water will remove the DNA from the binding columns). The columns are centrifuged at 11,000rpm for 1 min to elute the DNA. The eluted DNA was placed into the columns and centrifuged at 11,000rpm for 1 min.

## HCV DNA Sequencing

## Ethanol Precipitation

For each sample, a 0.5ml tube was labeled. Following manufacturer's instructions, a fresh stop solution/glycogen mixture was prepared. Five (5) µl of the stop solution/glycogen mixture was added to each of the labeled tubes. The sequencing reaction was transferred into each of the labeled tubes and mixed thoroughly. Sixty (60) µl cold 95% (v/v) ethanol was added into the tubes and mixed thoroughly. After that, it was immediately centrifuged at 14,000rpm at $4^0$C for 15 minutes.

The supernatant was carefully removed with a micropipette. The pellet was then rinsed with 200µL of cold 70% (v/v) ethanol and centrifuged for 2 minutes at 14,000rpm at $4^0$C. All the supernatant was carefully removed with a micropipette. It was vacuum dried for 10 minutes. The sample was re-suspended in 40µL of the sample loading solution (provided in the kit).

### Sample preparation for Loading into the Sequencing Instrument

The re-suspended samples were transferred to the appropriate wells of the sample plate. Each of the re-suspended samples was overlaid with one drop of mineral oil (provided in the kit). The sample plate was then loaded into the instrument and the sequencing program started.

### DNA Sequencing Procedure: Dye terminator cycle sequencing with quick start kit

Positive, purified PCR products were used as templates for sequencing in the Big-Dye Terminator V3.1 cycle sequencing kit (Applied Biosystems, Foster City, California, USA) reaction. While preparing the sequencing reactions all reagents were kept on ice. The reaction was prepared in a 2ml tube. The reaction volume contains 9.5µl of D.H2O, 2µl of primers, 10µl of DNA template and 8µl of DTCS Quick start master mix. The sequencing reaction in the PCR machine was set as $96^0$C for 20 seconds, $50^0$C for 20 seconds for 30 cycles and $60^0$C for 4 minutes. Samples were analyzed on an automated sequencer; ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, California, USA). Products were sequenced from both strands to get consensus sequences. The sequence from nt 9 to 252 (244 nt) was taken for analysis. A total of 9 isolates were sequenced in the 5′UTR region.

### Phylogenetic Analysis

DNA Sequence analysis was carried out to confirm the genotypes identified by nested-PCR analysis. The chromatographs were edited using Finch TV (version 1.4.0) software. All the nucleotide sequences obtained in this study were screened using the online BLAST (basic local alignment search tool) search tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to search for sequence similarities to previously reported sequences in the database. The classification of HCV genotypes and subtypes was according to the Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes (Simmonds *et. al.*, 2005). All isolates sequenced in the present study were aligned with the representative number of sequences for each major genotype and subtype selected from HCV database and Gene Bank using the Multiple Sequence Alignment Program, ClustalW (Markov *et al.*, 2009). Pair-wise comparisons for percent nucleotide homology and evolutionary distance were made. The phylogenetic analysis of HCV isolates was done with MEGA 6.0 software (Kumar *et al.*, 2015). Maximum composite likelihood algorithms were utilized, and phylogenetic trees were constructed using the neighbor-joining method (Saitou and Nei, 1987). Test of Phylogeny that is, the percentage replicate trees in which the associated taxa clustered together was performed with Bootstrap replication of 1000 and branch support values of >60%. The following are accession numbers of different HCV reference sequences used in this study; genotype 2b: EU256066.1, EU482877.1, AB691953.1, EU256078.1, KY780123.1 and EU155279.2.

### Data Analysis

Data obtained from questionnaire and the results of the laboratory analysis were analyzed using Statistical Package for Social Sciences Version 16.0. (SPSS Incorporation, 2007 Chicago, USA). Chi-Square test was employed to test for association between the variables and seroprevalence obtained at 95% confidence interval and a p value $\leq 0.05$ was considered significant.

## RESULTS

Plate I & 2: shows the amplicons obtained from the amplification of HCV RNA of the samples studied. The 5′UTR of the HCV genome region with 244bp was amplified using a nested PCR. Out of the 44 sero-positive samples amplified, 9 samples were PCR positive. The result showed the distribution of HCV RNA among participants with USNGH having the highest with 50% (N= 4), followed by GHK 14.3% (N= 2) and GHW 13.3% (N=2), the least from GHM 20% (N= 1) and there was no HCV RNA distribution in GHS as shown in table 1.

Nine (9) samples were successfully sequenced. The BLAST information obtained from our queried sequences and the closest corresponding sequences from the GenBank is shown on table 2. The result showed that samples 1, 7, 14, 19, 21, 30, 36, 78 and 121 all corresponded with genotype 1b. Figure 1 showed a common ancestral origin in the phylogenetic

relatedness of HCV isolates in the study with those from GenBank. Samples 1, 7, 19 and 21 were closely related to reference EU256066.1 (1b) Switzerland; sample 14 was closely related to EU256078.1 (1b) Switzerland, sample 30 was closely related to KY780123.1 (1b) Austria, sample 36 was closely related to EU155279.2 (1b) USA, sample78 was closely related to EU482877.1 (1b) USA and sample 121 was closely related to AB691953.1 (1b) Japan. The results obtained from the sequence analysis and alignment tree as shown in table 3 reveals that of the 9 samples sequenced, GHM, GHK, GHW and USNGHB all had genotype 1b respectively while GHS had none.

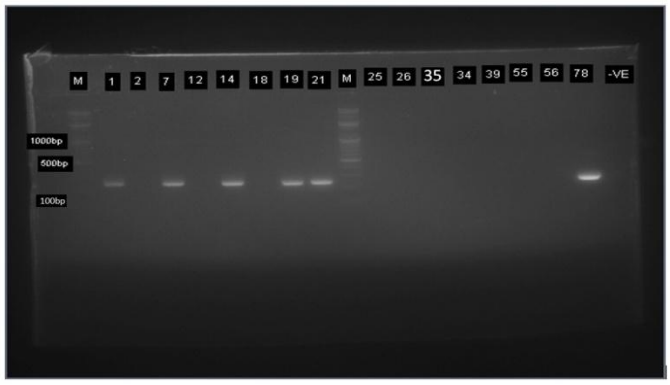


**Plate1**. Detection of HCV RNA in serum samples by Gel electrophoresis (positive samples are with band size of 244bps)

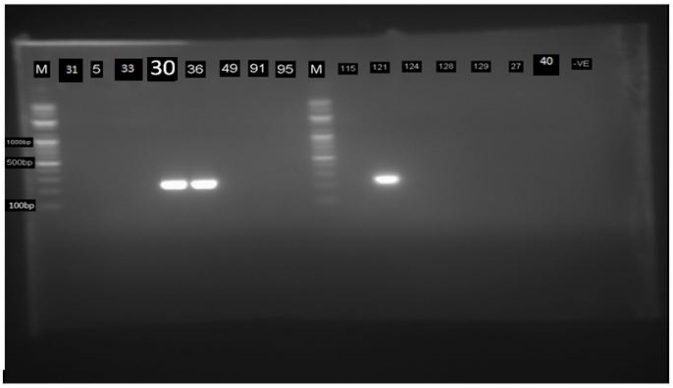


**Plate 2**. Detection of HCV RNA in serum samples by Gel electrophoresis (positive samples are with band size of 244bps)

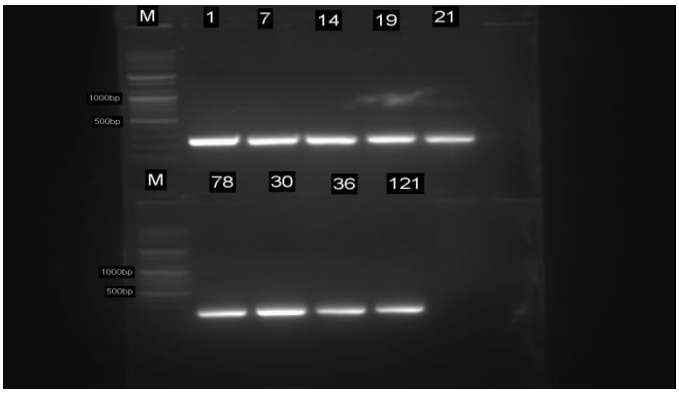


**Plate 3**. Detection of HCV RNA in serum samples as shown by Gel electrophoresis, Optimization reaction.

**Table 4.** Distribution of HCV RNA in HCV antibodies positive participants in the study area

| Location | number examined | number positive (%) |
|---|---|---|
| GHM | 5 | 1(20.0) |
| GHS | 2 | 0 (0.0) |
| GHK | 14 | 2 (14.3) |
| GHW | 15 | 2 (13.3) |
| USNGHB | 8 | 4 (50.0) |
| Total | 44 | 9 (20.5) |
| $\chi^2 = 5.6019$ | $df = 4$ | $p = 0.23091$ |

**Table 5:** Blast results of the 9 sequences

| Sample | Source | Length | Score | E-value | Identities | *Inference |
|---|---|---|---|---|---|---|
| 1 | HCV subtype 1b 5'UTR | 202 | 366 | 1e-97 | 100 | Subtype 1b |
| 7 | HCV subtype 1b 5'UTR | 185 | 311 | 5e-81 | 97 | Subtype 1b |
| 14 | HCV subtype 1b 5'UTR | 201 | 339 | 3e-89 | 97 | Subtype 1b |
| 19 | HCV subtype 1b 5'UTR | 201 | 355 | 3e-94 | 99 | Subtype 1b |
| 21 | HCV subtype 1b 5'UTR | 202 | 364 | 4e-97 | 99 | Subtype 1b |
| 30 | HCV subtype 1b 5'UTR | 200 | 361 | 5e-96 | 99 | Subtype 1b |
| 36 | HCV subtype 1b 5'UTR | 200 | 364 | 4e-97 | 99 | Subtype 1b |
| 78 | HCV subtype 1b 5'UTR | 201 | 357 | 7e-97 | 99 | Subtype 1b |
| 121 | HCV subtype 1b 5'UTR | 201 | 359 | 2e-95 | 99 | Subtype 1b |

** The query sequences covers 100%*

**Table 6:** Distribution of HCV Genotypes and Subtypes in the study population based on location

| | Location | | | | | |
|---|---|---|---|---|---|---|
| | GHM | GHS | GHW | GHK | USNGHB | Total (%) |
| Genotypes/subtype | | | | | | |
| 1b | 1 | - | 2 | 2 | 4 | 9(100) |
| Others | - | - | - | - | - | 0(0) |
| **Total** | **1** | **-** | **2** | **2** | **4** | **9(100)** |

***KEYS**: GHM = General Hospital Minna; GHS = General Hospital Suleja; GHW = General Hospital Wushishi; GHK = General Hospital Kotongora; USNGHB = Umaru Sanda Ndayako General Hospital Bida*

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 8.97702647 is shown. The tree is drawn to scale, with branch

lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree.
The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 15 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 184 positions in the final dataset. Isolated sequences and reference strains are represented by blue and red colours respectively. Evolutionary analyses were conducted in MEGA6.

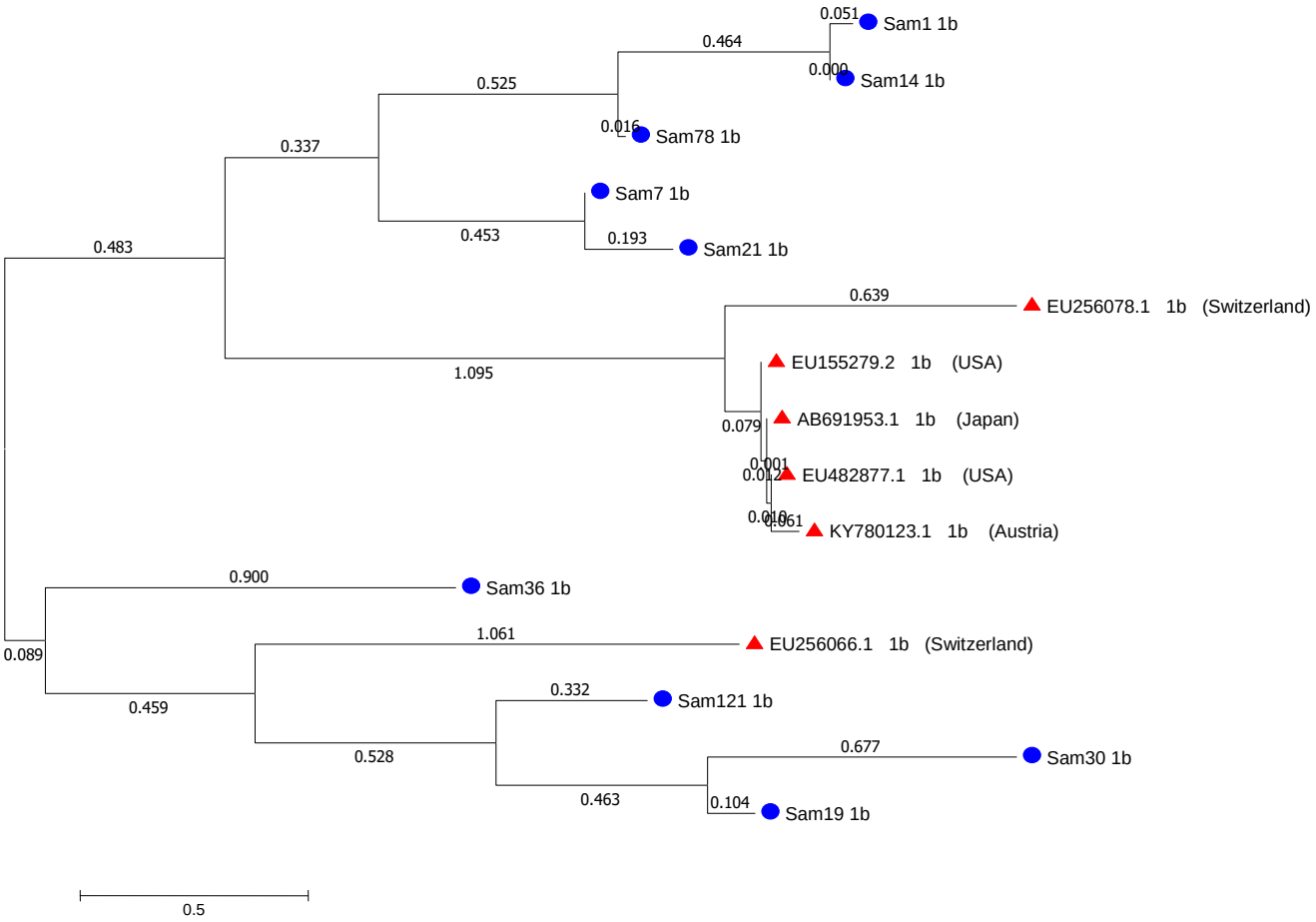


**Figure 1:** Evolutionary relationships of taxa

## DISCUSSION

Of 44 samples amplified with PCR, nine were positive, which mean not all sero-positive samples can be positive for PCR which is in agreement with the research of Pawlotsky [16] who studied 350 ELISA positive samples and 6 were positive for PCR. Bukh *et al.* [17] also reported on evaluation of 79 samples that were EIA positive and for which HCV RNA was not detected by PCR, which he attributed to low HCV nucleic acid concentration or HCV titer in the samples to be detected and also infection clearance and that individuals may just be carriers and virus is not actively replicating in them. The samples positive for PCR, implies that the individual has an active or ongoing infection and there is active viremia. [18, 19] Active viremia is an evidence of the replication of the virus and sometimes an indication of chronic HCV infection. Although, not all anti HCV positive are PCR positive because the level of viremia may be too low for detection by the current PCR assay,

poor storage of samples and treatment of patients may also have deleterious effect on PCR result. [20, 21]

The detection of HCV RNA by RT-PCR gives direct detection of the presence of the virus and also detection during seronegative window period, immediately after infection, therefore showing that the detection of HCV RNA may be more reliable than serology especially in patients who do not mount enough antibody response due to impaired immune system. [22, 23]

The sequence/phylogenetic analysis in this study using the amplicon products from the 5'UTR region of the HCV genome reveals the distribution of HCV genotype 1b subtypes in Niger State, Nigeria. Phylogenetic analysis of this region showed that the different genotypes circulating among patients clustered with similar strains from other countries in the world as shown on the phylogenetic trees in (Figure 1). The findings from this study are in support of previous reports by Sheyin *et al.* [12] and Shenge *et al.*[11] who reported genotype 1b subtypes in Kaduna and Ibadan, Nigeria respectively. This is contrary to findings by Oni and Harrison [10] in a pilot survey of HCV infection in Southwestern Nigeria who documented genotypes 1 and 4 in circulation in the area while Forbi *et al.* [8] reported genotypes 1 and 2 were identified in two remote villages in North-Central Nigeria. These show that even within regions in Nigeria, HCV genotypes are differentially distributed and this has serious implication for treatment of HCV infection in the country. The presence of diverse forms of HCV in Africa with emphasis on Nigeria lacking adequate data was reported by Oni & Harrison.[10] This diverse HCV types could lead to high treatment failure as well as high prevalence of liver disease including hepatocellular carcinoma (liver cancer) in chronically infected patients.[24] It may also constitute a serious obstacle to designing a universal vaccine against HCV infection. Medhat *et al.* [25] and Nie *et al.* [26] reported the circulation of genotypes 1 & 4 in Saudi Arabia and genotypes 2a, 3a, 6n, & 6a in China province respectively. Messina *et al.*[15] documented that genotypes 1, 2, and 3 have a worldwide distribution and HCV subtypes 1a and 1b are the most common prevalent genotypes. In this study, genotypes 2, 3, 4, 5 and 6 were not found and this further supports the fact that there are regional differences in the global distribution of the types of HCV genotypes.

Isolates 1, 7, 19 and 21 (Figure 1) showed high homology of 100%, 97%, 99% and 99% identities respectively with the reference strain EU256066.1 from USA which were identified as genotype 1b. [27] Isolate from sample 78 indicated 99% identity with reference isolate EU482877.1 which was identified as genotype 1b of USA sequence. [27] The phylogenetic tree showed that they are clustered into one branch.

Similarly, isolate 36 showed a high relatedness of 99% identity to reference EU155279.2 which was identified as genotype 1b of USA [27;] this showed that the isolate may be USA origin. Isolate 30 showed 99% identity with reference isolate KY780123.1 which was identified as genotype 1b from Austria. [28] Isolate 14 indicated 97% relatedness to reference EU256078 identified as genotype 1b from Switzerland [27] while isolate 121 was closely related to reference AB691953 with 99% identity which was identified as 1b from Japan. [29]

The diversity of HCV sequences remains a major obstacle for the development of effective vaccines and therapies.[30] HCV diversity is also an important factor in the response to antiviral therapy since genotype 1 is less responsive to IFN-α than genotype 2 and 3. [31, 32]

Knowing the infecting genotype has a direct impact on the prognosis and on the choice and duration of the treatment algorithm as well as being a statistically significant predictor of sustained virological response to antiviral therapy.[33] Based on the result of the HCV genotypes obtained in this study, clinicians and health workers in the study area can find the data useful in managing patients who are infected with the infection and also in creating awareness on the prevention and control of the infection to the general public, blood donors, pregnant women on antenatal care and other patients coming for health care in their facilities.

Although the HCV subtype afflicting a patient is not currently used to make clinical treatment decisions, knowing the viral subtype is important for studies of its origin, transmission, and evolution.[34, 35] For example, new emerging variants can be characterized better when they can be assigned an unequivocal subtype classification. Molecular epidemiology analyses rely on information about sequence variation at the subtype level. [36, 37, 38]The clinical usage of antiviral drugs could be guided by the HCV genotype.[39]

## CONCLUSION

Out of the 44 HCV antibodies positive samples obtained in the study, only 9 were PCR positive which showed that sero-positive samples may not definitely be positive for RT- PCR test. The nucleic acid technology if adopted for use in our

blood banks will reduce the transmission of HCV and other blood-borne pathogens through blood transfusion. The HCV genotypes detected in this study by sequence and phylogenetic analysis was genotypes 1b. All the genotypes determined were from the same ancestral HCV strain. There were high homogeneities between the genotypes determined in this study with earlier strains from United States of America, Japan, Austria and Switzerland. The result of the genotypes is important in the management of people infected with the infection.

## RECOMMENDATION

Although, both PCR and serology could be used to detect hepatitis C infection, PCR is more sensitive and should be carried out for individuals. The people who are positive for serology tests should further be tested for PCR and sequencing done to determine the genotype for effective management because response to treatment varies according to genotype. There should be commitment at the different levels of the government on the fight against the infection such as the establishment of standard diagnostic and research laboratories, provision of stable power, subsidizing or provision of free treatment for all patients with viral Hepatitis, funding of research works on HCV and the implementation of the findings.

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