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MOLECULAR DETECTION OF HEPATITIS C VIRUS AMONG BLOOD DONORS AND DRUG ADDICTS IN JOS, NORTH CENTRAL, NIGERIA

S.L. Gushit[1*] B.R. Alkali[2] K. Mohammed[3] E.I. Ikeh[4] T. Princewill[5]

Corresponding Authours: S.L. Gushit, Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. *E-mail-sethgushit@gmail.com. G.S.M. Number: +2348065642241.*

B.R. Alkali, Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. E-mail-balkali@yahoo.co.uk

K. Mohammed, Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

E.I. Ikeh, Department of Medical Microbiology, University of Jos , Jos Nigeria.

T. Princewill, Department of Medical Microbiology, University of Jos , Jos Nigeria.

ABSTRACT

Background: Hepatitis C virus infection is a global health problem associated with high morbidity and mortality rate. Prompt diagnosis of HCV is important for early detection, treatment and management of this disease. **Aim:** This study evaluated PCR-based detection method of HCV among blood donors and drugs addicts in Jos, Nigeria. Methodology: A total of 100 venous blood samples were examined from August 2018 to December 2018. **Results:** Of the overall blood samples, 15(15.0%) tested positive by PCR-Based detection method. Four with primary school education, 4(44.4%) and two with tertiary education 2(11.7%) tested positive by PCR,(P=0.074) which makes it insignificant. PCR recorded 15% among drug addicts and also zero percent among blood donors with 65.2% sensitivity and 65.2% Negative Predictive Value (NPV). The Chi-Square test shows hepatitis C virus infection is more associated with drug addicts than blood donors. **Conclusion:** The findings from this study concluded that, HCV is very prevalent among drug addicts in Jos and PCR method is recommended as a gold standard for effective detection of HCV infection in Jos, Plateau state, Nigeria.

Keywords: Molecular study, Hepatitis C Virus, PCR, Blood Donors, Drug addicts, Jos, North Central Nigeria.

INTRODUCTION

The word hepatitis comes from the ancient Greek word *hepar* (root word *hepat*) meaning liver and the Latin word (*itis*) meaning inflammation [(14)]. Hepatitis means injury to the liver with inflammation of the liver cells [(14)]. Hepatitis C virus is an infectious disease caused by hepatitis C virus (HCV) that primarily affects the liver [(22)]. During the initial infection people often have mild or no symptoms. Occasionally, a fever, dark urine, abdominal pain, and yellow tinged skin occur. The virus persists in the liver in about 75-85% of those initially infected. Early or chronic infection typically leads to liver cirrhosis. In some cases, those with cirrhosis will develop complications such as liver failure, liver cancer, or esophageal and gastric varices [(15)].

The hepatitis C virus (HCV) is a small enveloped, single-stranded, positive-sense RNA virus. It is a member of the hepacivirus genus in the family Flaviviridae. The half life of the virus particles in the serum is around 3 hours and may be as short as 45minutes. In addition to replicating in the liver the virus can multiply in lymphocytes [21].

HCV is spread primarily by blood-to-blood contact associated with intravenous drug use, poorly sterilized Medical equipment, needle stick injuries in healthcare, and transfusions. The virus may also be spread from infected mother to her baby during birth. Hepatitis C virus is among the most common cause of viral hepatitis which is considered as a major public health problem worldwide [4]. In 1999, it was estimated that 150-200 million people, approximately 3% of the world's population are living with chronic hepatitis C viral infection. Also about 3-4 million people die yearly from hepatitis C- related diseases. In 2010, HCV cases account for an estimated annual death of 16,000 people worldwide while 196,000 deaths occurs as a result of liver cancer, secondary to hepatitis infection [29]. There is a significant number of drugs addicts which comprises of both men and women of ages 18 to 60years who are indulged in the use of drugs in Plateau state [3].

MATERIALS AND METHODS

Study area

The study was carried out in Jos, Plateau State, North Central Nigeria from June to December 2018. It is situated along latitude 80°24N, longitude 80° 38E of the equator and the indigenous occupants of Jos are mostly Berom, Afizere and the Anaguta. Its average temperature is 18°c and annual rainfall of about 146cm (57in), Report from 2007, National Population Commission (NPC, 2006) [18].

Study design

This cross-sectional study is designed to detect hepatitis C virus among blood donors and drug users in Jos, Plateau State, North Central Nigeria using a valid and reliable structured administered questionnaire that has been tested and criticized by people and individual. The result was presented in tables, graphs, and figures.

Study population

In this study, one hundred (100) blood donors and drug addicts of ≥18 and above in Jos who have consented and voluntarily agreed to participate was recruited for the study.

Inclusion criteria

The inclusion criteria include; all blood donors and drug addicts between age 18 to 60 years, who were willing to give a written informed consent to participate in the study after counseling, was included in the study.

Exclusion criteria

Non- blood donors and non- drugs addicts, subjects < 18 years, subjects with known cases of hepatitis as a result of other causes, and those who refuse to give a written informed consent after counseling was excluded in this study.

Ethical consideration

Ethical approval was obtained from the Ethics and Research Committee of Plateau State Ministry of Health Jos (Appendix I), before commencement of the study and patients consent sought before enrolment. Informed consent for inclusion into the study was obtained from subjects who were willing to participate in the study (Appendix II). Structured questionnaire was administered to all eligible study participants to enable them fill their demographic, clinical and socio-economic details (age, sex and educational qualification) (Appendix I).

Sample size determination

The sample size was determined using the formula described by THRUSFIELD (1997);[27]

$$N = \frac{(1.96)^2.Pexp\ (1-Pexp)}{d^2}$$

where:-

N = Minimum required sample size

$(1.96)^2$ = Standard Normal deviation.

p = Population of success or prevalence (6.0%) [9].

d^2 = Tolerable margin of error or desired absolute precision of 5%.

Therefore:

$$N = \frac{(1.96)^2 \times 0.06 \times (1-0.06)}{(0.05)^2} = 84.$$

Attrition rate=10% of the minimum sample size;

Therefore; $\frac{10}{100}$x84=8.4=9

The minimum sample size was 93 but was increased to 100 to have more chances of precision and accuracy of the study. This minimum sample size was shared equally and fifty (50) blood samples were collected from both subjects.

Sampling method

Convenient sampling method was used to recruit all subjects of either sex between age group 18-60years that meet up the inclusion criteria was enrolled in this study.

Specimen collection

Patients' identity was confirmed prior to collection of blood sample. Powder free gloves were worn and tourniquet applied above the forearm followed by selection of suitable vein. The bleeding site was cleaned with 70% Alcohol swab and air dried. 3mls (3ml volume) of blood was drowned into a labeled clean anticoagulant (EDTA) tube and was nixed by inverting the tube 15 times. The anticoagulated tubes were assembled on the tubes rack System. The tube was labeled with patients name, age/sex, date of collection, time of collection and the tourniquet loosened and removed. Dry cotton wool was placed on the puncture site with little application of pressure on the bleeding site over a dry cotton wool with finger/thumb for bleeding to stop. The needle was then removed and discarded into the sharp bin. The samples were packed into tube rack and place in specimen transport box to be transported to the laboratory for analysis along with completed laboratory form and sample manifest. Sample tubes were not opened until samples are ready to be processed in the laboratory. The sample was transported to the laboratory at room temperature and process within 6hours of arrival [(1)].

Plasma separation from whole blood

The sample was centrifuged at 900-1800rpm for 20 minutes at room temperature to separates solids from liquids from packed cell using rotational forces. The plasma was separated from whole blood within 6 hours in the laboratory and 1.2ml of the plasma was aliquoted into 3 screw capped PCR- tubes [(31)].

Molecular analysis

TRIzol RNA extraction method

This is the process for isolation of total RNA by TRIzol Reagent. The RNA was isolated by TRIzol reagent (TRI REAGENT) which is a ready- to-use reagent for isolation of total RNA from cells and tissues. It is a mono-phasic solution of phenol and guanidine isothiocyanate, an improvement to single- step RNA isolation method. During sample homogenization or lyses, TRIzol Reagent maintains the integrity of RNA, while disrupting cells and dissolving cells components. Addition of chloroform followed by centrifugation separates the solution into an acqueous phase and an organic phase. RNA remains exclusively in the aqueous phase. After transfer of the aqueous phase, the RNA is recovered by precipitation with isopropyl alcohol. After removal of the aqueous phase, the DNA and proteins in the sample can be recovered by sequential precipitation. Precipitation with ethanol yields DNA from the interphase, and an additional precipitation with isopropyl alcohol yieldsproteins from the organic phase. TRIzol is made up of three (Tri) chemical reagents which include guanidine thioacyanate, phenol and chloroform. The mixture of guanidine thioacyanate and phenol was effectively dissolves DNA, RNA and protein on homogenization or lysis of tissue sample [(23)].

Lysis

The cells was lysed by adding I ml of TRIzol Reagent to I ml of previously prepared blood samples (5-10 $\times 10^6$cells). The mixture was then vortex for 10 seconds and incubated at 370C for 15 minutes. 500ul of phenol/chloroform (1:1) were then added into the mixture, vortexed for 1 minute and then incubated at 56^{o}C for 15 minutes. The tubes were then vortexed for 1 minutes and centrifuged for 3 minutes at 12000 rpm. The upper acqueous phase containing the viral RNA was carefully removed and placed in a clean eppendorf tube. 500ul Ice-cold isopropyl alcohol was added to the recovered acqueous upper phase, vortex for 30 seconds and centrifuged for 5 minutes at 12000 rpm (Appendix I) [(23)].

Precipitation

When the lyses step was completed, the aqueous phase which contain the RNA was transferred into a fresh tube and precipitated by mixing with isopropyl alcohol of 0.5 ml per 1 ml of TRIzol Reagent used for the initial homogenization (Appendix II). It was well mixed by inverting the tube several times. It was then incubated at 15 to 30^{o}C for 10minutes and centrifuged at 12,000 × g for 10minutes at 4^{0}C. The RNA precipitate was invisible before centrifugation but forms a gel-like pellet on the side and bottom of the tubes after centrifugation [(23)].

Purification

Now that RNA has been separated from the aqueous phase, it was rinsed with alcohol to remove any remaining unwanted material and cellular debris. At this point, the purified RNA was re-dissolved in water for easy handling and storage [(12)].

Sample preparation

The RNA was isolated by centrifuging at 12000rpm for 10minutes and the cells were lysed in 1ml TRIzolper 5-10X10^{6} of blood cells.

Homogenization

Tissue samples were homogenized in a Polytron homogenizer and the insoluble materials were removed from the homogenate by centrifuging at 12,000 ×g for 10minutes at 2 to 8 ^{0}C with DEPC treated water, absolute ethanol and 2ml of TRIzol in a 5ml tube to clean the probe before use. Probe was washed with DEPC treated water and absolute ethanol between samples. Probe was inspected for residual tissue. It was dissociated according to the manufacturer's manual, the probe was cleaned thoroughly with DEPC treated water and absolute ethanol. It can be stored at -70^{0} c after use. Samples were allowed to stand for 5 minutes at room temperature for complete dissociation of nucleoprotein complexes and the cleared homogenate solution was transferred into a fresh tube and proceeds with chloroform and phase separation [(12)].

RNA separation

The homogenized samples was incubated for 5 minutes at 30 ^{0}C to allow complete dissociation of nucleoprotein complexes and 0.2 ml of chloroform was added to 1ml of TRIzol Reagent. Sample tubes were capped firmly to avoid spillage. Samples were vigorously vortex for 15 seconds and incubated at room temperature for 2 to 3 minutes. Samples were centrifuged at 12,000 ×g for 10 minutes at 4 ^{0}C. Following centrifugation, the mixture was separated into 3 layers: Lower yellow, phenol-chloroform layer containing the protein, and an inter-phase containing the DNA, Colorless upper aqueous layer containing RNA (Appendix I). Upper aqueous layer was carefully transferred into a fresh tube. The Volume of the aqueous layer (usually 60% volume of TRIzol Reagent used for homogenization) was measured.200µl pipette was used to avoid the inter-phase contamination.700µl of a colorless layer was transferred into a clear tube. The inter-phase and organic layer was stored at 4^{0}c for subsequent DNA and proteins isolation [(26)].

RNA precipitation

The RNA was precipitated by mixing with 0.5 ml of isopropyl alcohol per1ml of TRIzolReagent used for the initial homogenization. Samples were well mixed by inverting the tubes several times and incubated at 30 ^{o}C for 10 minutes. It was centrifuged at 12,000×g for 10 minutes at 4 ^{o}C [(29)]. The RNA precipitate was invisible before centrifugation but forms gel-like pellet on the side and bottom of the tube after centrifugation (Plate 1.7).

RNA wash

The supernatant was carefully discarded and RNA pellet was washed by adding 1 ml of 75% ethanol in RNA free water. Samples were mixed by vortexing and centrifuged at 12,000 x g for 2minutes at 4 ^{o}C. The above washing procedure was repeated once to completely remove

the leftover ethanol. The water in the ethanol was used to wash the salts out of the RNA and was stored in ethanol at -20^{0}C [6].

Redissolving RNA

RNA pellets were allowed to air-dry for 5-10 minutes and was not dried by centrifuge under vacuum. The RNA pellet was not completely dry to avoid decrease in its solubility.RNA was dissolved in dethylpyrocarbonate-treated water by passing solution a few times through a pipette tip and incubated for 10minutes at 55 to 60^{0}C [6].

Reverse Transcription PCR (RT-PCR) this is a method used to amplify, isolate and identify a known sequence from a cellular or tissue RNA. The PCR is preceded by a reaction using an enzyme called Reverse Transcriptase to convert RNA to cDNA followed by the amplification of the newly synthesized cDNA by standard PCR procedures. It is synthesized in a reaction that is catalyzed by the reverse transcriptase and DNA polymerase enzymes. It is also a process in which a double stranded DNA molecules are made from a single stranded RNA. The name of this method is formed by its opposite direction to transcription. It also involves the presence of a reverse transcriptase enzyme, a primer, DNTAs and a RNase inhibitor [2].

Principle

The principle is based on an enzymatic reverse transcription of RNA genomes into DNA by integrating it into the host DNA genome which replicates along with it. Reverse transcription originate from the concept of reverse transcriptase found and isolated from reverse-transcribing viruses which use the enzyme to reverse-transcribe their RNA genomes into DNA [2].

Oligonucleotide primers

Primers designed by Murphy *et al.*, 2007 for sequence analysis of the NS5B region for routine genotyping of Hepatitis C Virus with reference to C/E1 and 5′ Untranslated Region Sequences (5’ UTR) was modified and adopted in this study. Nucleotide sequence analysis of the NS5B region was performed to identify genotypes of 100 hepatitis C virus (HCV) RNA-positive plasma samples of blood donors and drug addicts collected in Jos, Plateau State, Nigeria [17].

Table 1: Murphy primers for HCV genome detection

Genomic region and Primer	Polarity	Sequence	Position	Size of PCR product (bp)
5’UTR				
DM50	Antisense	5’- CTC GCA ACG ACC CTA TCA GG-3’	292-311	241

DM51	Sense	5'-GAA AGC GTC TAG CCA TGG CGT TAG T-3'	71-95

HCV genome detection [17]

HCV genome amplification.

One hundred (100) HCV RNA plasma samples from drug addicts and blood donors were collected and analysed in this study. Gene-specific HCV primer pair of DM50/DM51 proved to be efficient in detecting the HCV genotypes and generating the HCV amplicons region of C and E1 in the 5′UTR at 241bp respectively. The 5′ untranslated region (5′UTR) is the region of choice for qualitative HCV RNA detection due to its high level of conservation and sensitivity. Of the 100 studied subjects, 15 were positive to HCV genotype. The HCV plasma samples files for the sense and antisense sequencing reactions were electronically transferred via an internal network to a PCR platform [17].

Procedure for transcription

Each component were mixed and briefly centrifuged in 200µl PCR tube as shown in table 2.

Table 2: Component of HCV RNA transcription

Component	Volume
RNA sample	(≤5µg total RNA)
Primer	1µl
(50µM oligo dT or 2µM gene-specific primer(GSP) 10mM dNTP	1µl
DEPC-treated water	to 10µl
incubated for 5 minutes at 65°Cand place on ice for 1minute.	

Component of transcription.

Procedure for cDNA synthesis

The cDNA synthesis solution was mixed in the following order as shown in table 2

Table 3.3: Component of cDNA synthesis solution

Component	Volume
10X RT buffer	2µl
25mM MgCl2	4µl
0.1M DTT	2µl
RNase Inhibitor	40U
Reverse Transcriptase	200U

The combine 10µl cDNA synthesis solution, RNA sample and primers were gently mixed and centrifuged. Oligo (dT) or GSP was incubated for 50minutes at 50°C while random hexamer was incubated for 10minutes at 25°C and then 50minutes at 50°C.The mixture was incubated at 85°C for 5minutes and then chill on ice.1µl RNase H was added to each tube and centrifuge. It was then incubate at 37°C for 20minutes. At this stage, the cDNA is synthesized and was preceded to PCR immediately or could either be stored at -20°C [2].

PCR procedure

The cDNA was taken through series of cyclic reactions conducted in an automated, self-contained thermo cycler machine.The major three main steps involves in PCR techniques are; Denaturation, Annealing and Extension [33].
The cDNA was pre-heated to 94-96^{o}C for 10minutes to initiate thereaction and when the temperature was set at 94-96^{o}C for 1 to 5minutes, the cDNA was denatured and the double stranded cDNA bond was separated into 2 single strands.
The PCR temperature was then lowered to 58-60°C for 1minute and the primers anneal to their complementary sequence on the cDNA template. At 72-80°C (most commonly 72°C). The cDNA templates increased exponentially in a 5′ to 3′ direction by the action of DNA polymerases.
The thermo cycler was heated to 70-74°C for 5-15 minutes for full extension of any remaining single-stranded cDNA and the whole process of PCR cycle stopped at 4°C.The PCR product was obtained and electrophoresised on agarose gel, photograph and analyse [19].

Agarose gel electrophoresis

Gel electrophoresis is the standard laboratory procedure for separating DNA by size (e.g., length in base pairs) for visualization and purification. Electrophoresis uses an electrical field to move the negatively charged DNA through an agarose gel matrix toward a positive electrode. Shorter DNA fragments migrate through the gel more quickly than longer ones[23]. Thus, you can determine the approximate length of a DNA fragment by running it on an agarose gel alongside a DNA ladder (a collection of DNA fragments of known lengths).

Pouring a standard 1% agarose gel

One gram (1g) of agarose was measured. (Agarose gels are commonly used in concentrations of 0.7% to 2% depending on the size of bands needed to be separated). The mass of agarose was adjusted in a given volume to make gels concentration of 1 g of agarose in 100 ml of TBE will make a 1% gel. Agarose powder was mixed with 100 ml 1xTBE in a microwavable flask. It was then microwave for 1-3 min to dissolved agarose completely and the solution was not over boiled. It could be microwave in pulses, swirling the flask occasionally as the solution heats up. It was microwave for 45 seconds, stops and swirl then continues towards boiling and kept under watch to avoid boil over. It was then allowed to cool for 5minutes to bring down the temperature to about 50 °C and ethidium bromide (EtBr) was added to the final concentration of approximately 0.2-0.5 µg/ml (usually about 2-3 µl of lab stock solution per 100 ml gel). EtBr binds to the DNA and allows visualization of the DNA under ultraviolet (UV) light. Agarose was poured slowly into a gel tray with the well comb in place to avoid bubbles disrupting the gel and was allowed to stand at room temperature for 30 minutes to completely solidified. The gel was soaked in EtBr solution and rinses it in water before imaging [7].

Samples loading and running on agarose gel

Loading buffer was added to each DNA samples to aids DNA migration and to increase the density of DNA sample causing it to settle at the bottom of the gel well, instead of diffusing in the buffer. After it was solidified, the agarose gel was placed into the gel box (electrophoresis unit) and the gel box was filled with 1xTBE until the gel was covered and a molecular weight ladder was carefully loaded into the first lane of the gel. Samples were carefully loaded into the additional wells of the gel and run at 90 V for 45 minutes and the dye line was approximately 80% down the gel [31].

Note: Black is negative, red is positive. The DNA is negatively charged and run towards the positive electrode. The power source was turned off and the electrodes were disconnected from the power source, and the gel was carefully removed from the gel box. If EtBr were not added to the gel and buffer, then place the gel into a container filled with 100 ml of TBE running buffer and 5 µL of EtBr, and then place it on a rocker for 30 minutes, replace EtBr solution with water and destain for 5 minutes. Molecular Documentation device were used

visualize DNA fragments. The fragments of DNA are referred to as ‘bands’ due to their appearance on the gel [32].

Analyzing gel

Using the DNA ladder in the first lane as a guide and following manufacturer's instruction the size of each band of DNA on the sample lanes were inferred [12].

Determination of hepatitis C virus prevalence

PCR-Based method

Formula = TP/Total × 100,
Where;

TP = True Positive = 15

Total =100
15/100 × 100 = 15%

One hundred (100) subjects were tested for disease. 15 people have the disease; 85people are not diseased with the prevalence of 15.0% [16].

Sensitivity of the method

Sensitivity

Sensitivity is the probability of a test to indicate the presence of a 'disease' among those with the disease.

Sensitivity of PCR-Based method

Formula =Tp/Tp+FN× 100

Where;

TP = True positive = 15

FN = False Negative = 8

15/23 × 100 = 65.2%

The test has 65.2% sensitivity. In other words, 15 persons out of 85 persons with negative results are truly negative. Sensitivity is two-thirds and therefore it detects two-thirds of the people with the disease [16].

Negative predictive value (NPV) for PCR-Based method

Negative Predictive Value indicates the proportion of subjects who are not likely to have the disease if the test is negative.

Formula = TN/TN+FN× 100

Where;

TN = True Negative = 15

FN = False Negative = 8

15/23 × 100 = 65.2%

The Negative Predictive Value of PCR-BASED Method shows the subject has 65.2% chances of not having the infection [16].

Note: Positive Predictive Value (PPV) cannot be calculated because the variable analyzed in this study has no false positive results.

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS), version 23.0. (Inc.,Chicago, 111). The distribution of Hepatitis C virus infection among blood donors and drug addicts was presented in tables which shows infected and not infected variables. Pearson Chi-square (x^2) test was used to compare the prevalence of Hepatitis C virus infected variables against not infected variables by PCR method. Analysis for categorical variable was carried out using Chi-square test to determine the association. Values were considered statistically significant at $P<0.05$[22]. Frequency distribution table was used to obtain the prevalence of Hepatitis C virus infection among blood donors and drug addicts in Jos by PCR methods. Contingency table was used to calculate the sensitivity of PCR methods using this formula: TP/TP+FNX100 [16].

RESULTS

Result presentation

One hundred blood samples were collected from blood donors and drugs addicts in Jos, Plateau State and analyzed for hepatitis C virus infection. The results of the study are presented in tables as follows:

Of the 50 blood donors screened by PCR-Based detection method for hepatitis C virus infection, none tested positive to the virus (Table 4).

Fifty (50) drug addicts were screened by PCR-Based detection method for hepatitis C virus, 15 tested positive to HCV infection (Table 4.1).

Table 4: Overall prevalence of hepatitis C virus infection among blood donors and drug addicts in Jos, Nigeria.

n=100

Method **PCR**	**No. Examined**	**No. Positive**	**No. Negative**	**% Positive**	**% Negative**
BLOOD DONORS	50.0	0.0	50.0	0.0	100
DRUG ADDICTS	50.0	15.0	35.0	30.0	70.0
Total	100	15.0	85.0		

TP/Total × 100=15/100 x100 =15%

Table 4: Prevalence of hepatitis C virus infection among blood donors according to age-group, gender, educational and marital status using PCR-Based method. n=100

Variable	Infection n%	No infection n %	Total n %	*P-value*
Age Group (Year)				
20-30	0(0.0)	23(100)	23(23.0)	**
31-40	0(0.0)	17(100)	17(17.0)	
41-50	0(0.0)	6(100)	6(6.0)	
51-60	0(0.0)	3(100)	3(3.0)	
>60	0(0.0)	1(100)	1(1.0)	
Gender				
Male	0(0.0)	25(100)	25(25.0)	**
Female	0(0.0)	25(100)	25(25.0)	
Educational status				
Primary (1^0) education	0(0.0)	1(100)	1(1.0)	**
Secondary (2^0) education	0(0.0)	23(100)	23(23.0)	
Tertiary (3^0) education	0(0.0)	26(100)	26(26.0)	
Marital status				
Married	0(0.0)	24(100)	24(24.0)	**
Unmarried	0(0.0)	26(100)	26(26.0)	

Key: **= values cannot be computed.

Table 4.1: Prevalence of hepatitis C virus infection among drug addicts according to age-group, gender, educational and marital status using PCR-Based method.

Variable	Infected n %	No infection n %	Total n %	*P-value*
Age group (year)				
20-30	11(47.8)	12(52.2)	23(23.0)	0.334
31-40	4(23.5)	13(76.5)	17(17.0)	
41-50	0(0.0)	6(100)	6(6.0)	
51-60	0(0.0)	3(100)	3(3.0)	
>60	0(0.0)	1(100)	1(1.0)	

Gender				
Male	11(44.0)	14(56.0)	25(25.0)	0.071
Female	4(16.0)	21(84.0)	25(25.0)	
Educational status				
Primary (1^0) education	4(44.4)	5(55.6)	9(9.0)	0.074
Secondary (2^0) education	9(37.5)	15(62.5)	24(24.0)	
Tertiary (3^0) education	2(11.7)	15(88.2)	17(17.0)	
Marital status				
Married	4(25.0)	12(75.0)	16(16.0)	0.180
Unmarried	11(32.3)	23(67.6)	34(34.0)	

Pearson Chi-square was used

Table 2. Sensitivity of hepatitis C virus infection using PCR reagent by 2x2 contingency table.

Screening Test	**Infected**	**Not infected**	**Total**
Positive	True positive=TP	False positive=FP	TP+FP
	15	0	15
Negative	False negative=FP	True negative=TN	Total
	8	77	85
Total	TP +FP	FP+TN	TP+FP+FN+TN
100	23	77	100

Sensitivity= TP/TP+FN× 100/1=15/15+8x100/1=15/23x100/1= 65.2%

4.1.1. Analyzing gel

Using the DNA ladder in the first lane as a guide and following manufacturer's instruction the size of each band of DNA on the sample lanes were inferred as follows:

1. Representative gel profile of HCV positive samples for drug addicts on nested-PCR assay.

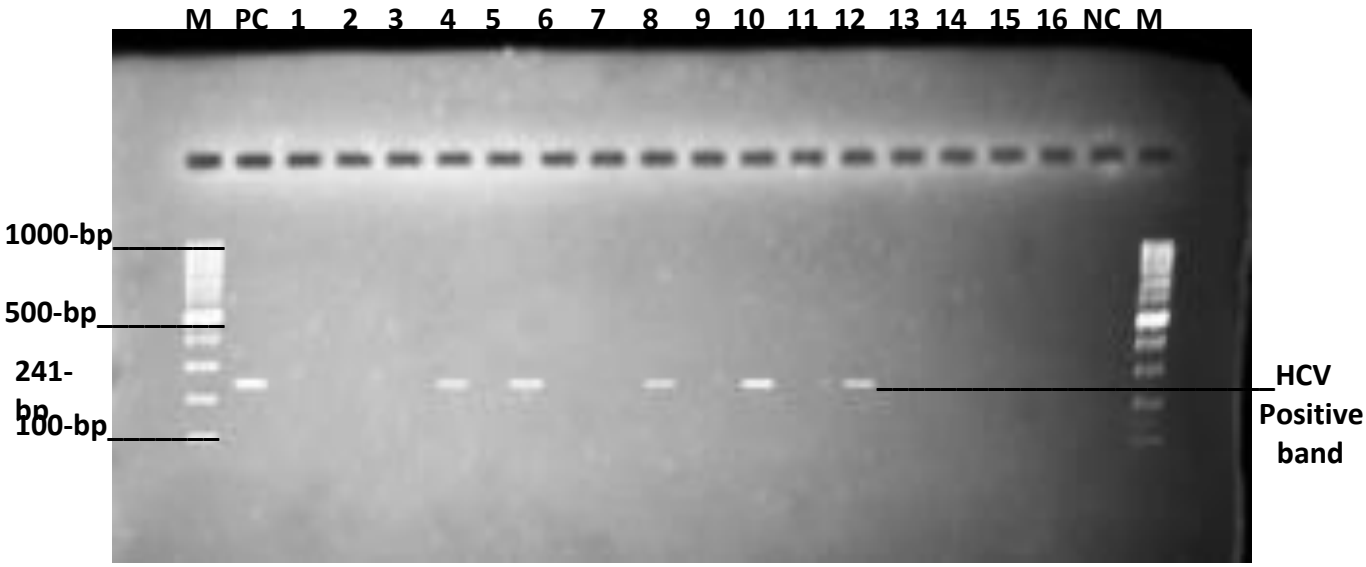

Plate I: Representative gel profile of HCV positive samples.
Key: Lane "M" (from left to right) shows DNA Markers or ladder for HCV with 1200-bp**. Lane "PC"** is the Positive control with 241-bp HCV Genotype-Specific band. Lane "**NC"** contained the Negative Control**.** The Drug Addicts gel had 16 wells. The 1st well labeled "1" contained sample number 001 for Drug Addicts in that order.

1. Representative gel profile of HCV negative samples for blood donors on nested- PCR assay.

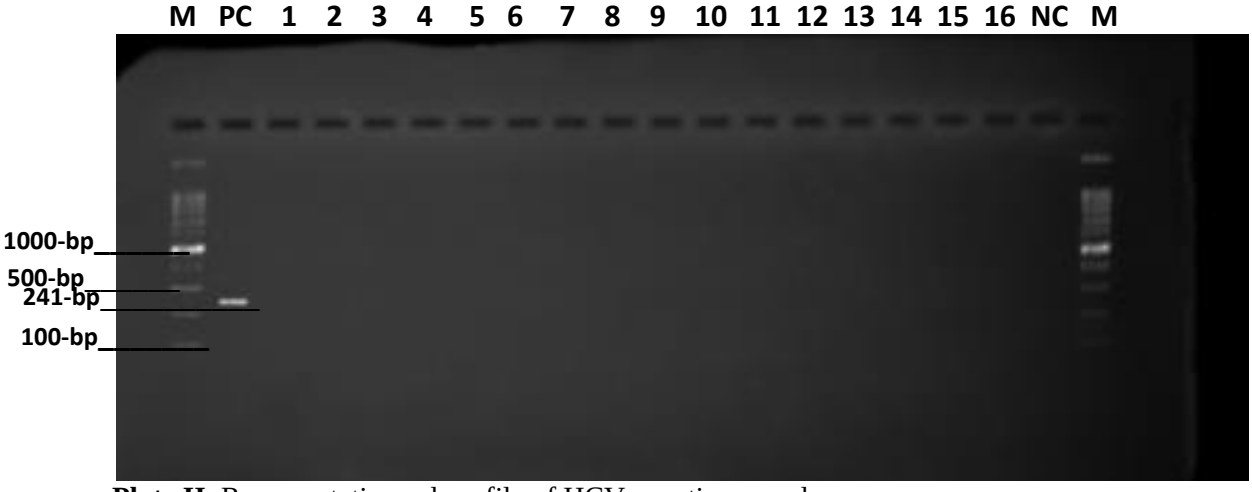

Plate II: Representative gel profile of HCV negative samples.

Key: Lane "M**"**(from left to right) shows DNA Markers or ladder for HCV with 1200-bp**. Lane "PC"** is the Positive control with 241-bp HCV Genotype-Specific band. Lane "**NC"** contained the Negative Control**.** The Blood Donors gel had 16 wells. The 1st well labeled "1" contained sample number 001 for Blood Donors in that order. **All Lanes** shows no HCV specific bands of 241-bp and these indicates that, all samples are negative to HCV virus.

Discussion

Infections due to Hepatitis C viruses (HCV) are significant health problems around the world. Globally, viral hepatitis is the commonest cause of hepatic dysfunction in people living with the virus. In this study, the prevalence of Hepatitis C virus among drugs addicts in Jos Plateau State North Central Nigeria was 15.0% with sensitivity and Negative Predictive Value of 65.2% was recorded by PCR-Based detection Method.

In most developing countries Nigeria inclusive, hepatitis C virus infections are mostly pronounce in certain groups of the populations. For example, 23% of new HCV infections and 33% of HCV mortality are common to injecting drug addicts. But unfortunately, these groups of people who inject drugs are not always included in national responses. In most of this countries infection control practices are insufficient and the infection is often rapidly spread to the general population [(25)].

In addition, PCR detects the DNA of the virus and not the antigen, which gives PCR an added advantage over other diagnostics methods which relies on antigen or antibodies for the detection of HCV. PCR also has an ability to detect HCV in subjects with low HCV concentration below the detection capacity of other diagnostic methods. Furthermore, because of this substantial proportion of HCV infections which were detected by PCR, the method therefore is sufficiently sensitive and should be encouraged for mass screening exercise and the most reliable method for the detection and confirmation test for HCV among patients. In addition, this study provides information about the necessity of training and retraining of healthcare professionals on how to adequately operates and manage the PCR machines for optimum productions of laboratory results to our patients. This study shows marked

differences in the performance of the method used on subjects studied (PCR method 65.2% sensitivity), this clearly revealed that the use of other diagnostics methods in the detection of HCV should not to be used as gold standard for the detection of hepatitis C virus in this modern days era and PCR should be made available, affordable and accessible to all healthcare facilities to both rural and urban communities in our country.

It was also observed in this study that, the highest prevalence rate of 47.8% by PCR was recorded in age group 20-30 years followed by age group 31-40 years with 23.5% prevalence with P=0.334 which makes it insignificant. This findings is in consistency with Odjimogho *et al.,* (2018) who reported 29.0%, 37.0% and 22.0% HCV prevalence between age groups 28-37years, 38-47years and 48-57years respectively, but in contrast with the reports of Itelima (2017) who recorded 2.5%, 2.0% and 0,2% within the same age groups intervals of 25-34years, 35-44years and >55years respectively and these has great implication on the government, family and the society at large because it causes dysfunctional patterns of use which constitute nuisance and dependency on government and the family. It also lead to high level of psychiatric disorder among the youths such as mood change, anxiety and personality disorder (antisocial) associated with drug addiction. These findings showed that, the vulnerable populations are the young among drug addicts and the older age groups are less vulnerable to the virus which could be due to their low engagement in risk behavior as compare with the younger age groups.

In this study, it was also observed that, PCR recorded the highest prevalence of 44.4%, of the drug addicts were among those with primary education followed by secondary education with the prevalence of 37.5% and the least of 11.7% prevalence were among those with tertiary education(P=0.074) which is insignificant. This study disagrees with the report of Odjimogho *et al.,* (2018) who reported the prevalence of HCV to be 6.20%, 32.20% and 60.30% among subjects with primary, secondary and tertiary education respectively. In this study, the highest prevalence were found to be among those with primary and secondary education which are most vulnerable to HCV which agrees with the findings of Awan *et al,* (2010) and this could

be as a results of habitual risk behavior, inadequate knowledge about the virus, ignorance, sharing of sharps objects, indiscriminate sexual intercourse with more than one partner.

The findings of the study observed the prevalence of 25.0% and 32.3% among married and unmarried drugs addicts with P-value of 0.180 which is insignificant. This findings is higher than the study carried out by Itelima (2017) which in her study, reported the prevalence of HCV to be 2.85% and 2.0% among married and unmarried subjects but in concert with Odjimogho *et al.,* (2018) who reported 40.40% and 20.10% prevalence of HCV among married and unmarried subjects respectively. The Chi-Square analysis shows that, hepatitis C virus infection is more common among drug addicts than blood donors in Jos, plateau state, Nigeria and this predisposes the country to high degree of morbidity and public health risk of HCV epidemic. The sensitivity of PCR were calculated using the formula: Sensitivity for PCR=TP/TP+FNx100/1=15/23x100/1=65.2% and the Negative Predictive Value (NPV) obtained for PCR was 65.2%. The Positive Predictive Value and the specificity cannot be calculated because there were no false positive values obtained in the observation the method used. The sensitivity of PCR is significant and considered a gold standard in HCV detection and a good screening test for hepatitis C virus infection.

These differences observed between this study and other studies previously conducted may be due to peculiarities in the route of HCV transmission, socio-cultural practices, environmental factors and also differences in methods of studies. Meanwhile, comparison between this study and others' should be noted because different methods might have been used by other researchers, but in this study, it was aimed to detect HCV using PCR-Based detection method where the DNA of this virus were detected rather than antibodies or antigen alone [(11)].

The prevalence of hepatitis C virus among drugs addicts recorded in this study is considered high in this part of the world and this could be as a result of genetical influence of drug addiction mostly pass by parents of drug addiction to their children, that is, apart from their young ones to physically learn the experience from them, the urge can also be inherited from their parents through genes. It has also been noted that drugs addiction often runs in families, suggesting genetics inclination is one of the causes of drug addiction. While having parents

that use drugs puts a child at risk, it is possible for the child to grow up with or without drug addiction problems, but in this sense, genetics alone is not the cause of drug addiction but in combination with poor relationship with parents, use of drugs by peers or friends, unwillingness to caution the young ones by parents in the use of drugs, poor parental upbringing which may lead to behavioral problems, academic pressure or poor achievement in school, approval of drug use in the school, peer group or community and availability of drugs from friends or peers. Drug addiction usually occurs in line with other disease conditions like mental illness. Although, mental illness might not lead to cause drug addiction, but a condition may predispose an individual to, and can be complicated by the other. One can get expose to drugs as a result of an attempt to manage the symptoms of other underlying disease conditions like mental illness. For example, a depressive person may repeatedly treat with drugs that can elevate his mood which can make him to get use to the drug and make him use to it [(9)]. The depression wasn't the primary cause of drug addiction, but a contributing factor. The presence of drugs in homes or in the environment inhabited by people can predispose and individuals especially the younger ones to the use of drugs which can serve as a major cause of drug addiction in certain life circumstances and can serve as risk factors rather than the direct cause of drug addiction. Parental use of drugs and neglect of their responsibility to check the activities of their children are common cause of drug addiction. The adolescent or pre-adolescent may try to gain attention from an inattentive parent or escape an abusive one by using drugs. Meanwhile, prolonged attempts by youths through drug use can be a cause of drug addiction [(28)].

Conclusion

This study confirms that, HCV is very prevalent among drug addicts in Jos plateau state, North central Nigeria. Couple with the insufficient information on hepatitis C virus infection, there is no doubt that, it is a major health problem which requires greater attention in Jos, plateau state, Nigeria. With the method used, PCR detected 15% among the drug addicts with 65.2% sensitivity and 65.2% NPV. This study finally concluded that, PCR-Based detection method is the gold standard for HCV detection and should be used by all health facilities in Jos, North Central, Nigeria. The drug addicts can serve as source of infection to the general

public in the country if not treated and this further confirms and recommends routine baseline screening for HCV markers in blood donors and drugs addicts in curtailing the spread of the infection.

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