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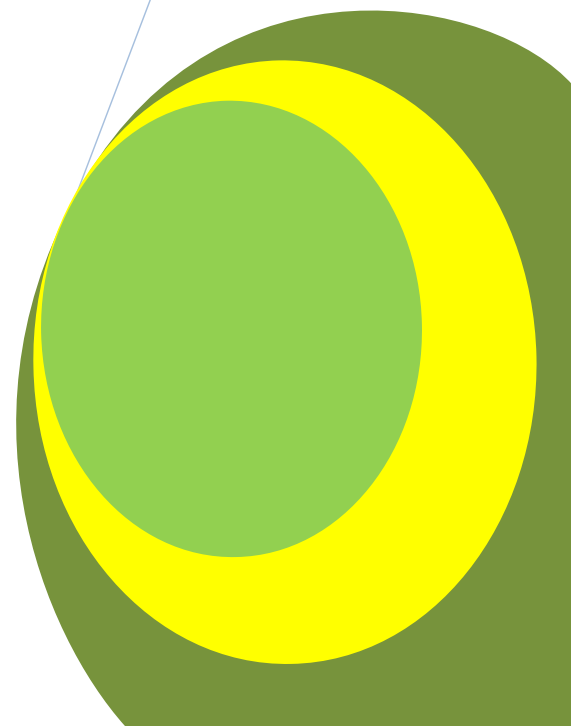
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Molecular Epidemiology of HCV Genotype in Relation to Viral Load of Infected Individuals in Northwestern Nigeria

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ABSTRACTS

The global burden of Hepatitis C Virus (HCV) is increasing and its believed to be one of the leading causes of hepatocellular carcinoma and a major cause of chronic liver disease. The present study aimed at evaluating the prevalence of HCV genotype in association with viral load of infected individuals. A total of one hundred and seventy three (173) HCV seropositive patients were included in the study. RNA extraction was performed using Accuprep RNA extraction kit according to the manufacturer's instructions. After running the HCV cDNA on 1.5% agarose gel, genotyping was completed using Beckman Coulter sequencing machine based on chain termination method. Accupower HCV-1111 (IVD) was used for the preparation of master mix and light cycler real time PCR machine version 5.2 was used for viral load. The result of the current study revealed that 93 (54%) patients were infected with the viral genotype 1b, followed by 1a with 64 (39%), 1c, 2a and 2b with 2 (1%), 9 (5%) and 5 (3%) respectively. Conclusively, genotype 1b has the highest prevalence, while 1c with lowest number of infected individuals. Moreover, in all the ranges of viral loads in the study, 1b was found to have highest prevalence.

Key Words: HCV, Viral load, Genotype.

1. INTRODUCTION

Hepatitis C Virus (HCV) is one of the number one causes of hepatocellular carcinoma and a major cause of chronic liver disease (Seeff *et al.*, 2001; Houghton 1996), it has a high mortality rate which account for approximately 350000 deaths of the infected people every year. It is estimated that about 3% of the world's population have HCV. There are about 4 million carriers in Europe alone (Jinget *et al.*, 2015). HCV is estimated to be ten times more infectious than Human Immunodeficiency virus (HIV) but less infectious than Hepatitis B virus (Seeff *et al.*, 2001). Hepatitis C virus was discovered in 1989 during the era of research aimed at finding the agent responsible for 80% of transfusion associated hepatitis cases other than hepatitis A and B which as of then was called non-A, non-B hepatitis and the presumed etiologic agent of non-A non-B hepatitis virus (Hsuet *et al.*, 1994; Alter 1999). HCV is positive sense single strand RNA virus of about 50nm in diameter belonging to the genus Hepacivirus and a member of the Falviviridae family (Mackay 2004; Hsuet *et al.*, 1994; Alter 1999). It is spherical, lipid enveloped with genome of about 9600 nucleotides and six major genotypes (1-6) have been identified to date (Tsukiyama-Kohara *et al.*, 1992). The genotypes differ by 31 to 34% in their nucleotide sequences based on their full-length genome sequence comparisons (Choo *et al.*, 1985; Simmonds *et al.*, 2005). Transmission is mostly by blood and blood products, individuals that use intravenous drugs are equally at risk of infection. In addition, vertical transmission and sex with an infected partner are also risk factors (Dusheiko *et al.*, 2005; Esfahani *et al.*, 2010). HCV infection is generally asymptomatic during the acute phase and about 85% of them become chronically infected. HCV therapy is genotype/subtype specific which makes the genotype identification crucial for clinicians when choosing chemotherapy, because these

genotypes have been reported to exhibit different responses to prescribed anti-viral therapies and require varying duration and doses of therapy (Jimenez-Mendez *et al.*, 2010). Its genotype is worldwide distributed but some strains are peculiar to some particular geographical regions, 1a, 1b and 3a genotypes are prevalent strains all over the world (Pawlotsky, 2003). Genotypes 1, 2 and 4 are confined to certain regions of Africa and the Middle East, whereas genotypes 3 and 6, divergent endemic strains are detected in numerous localities of Southeast Asia (Purcell, 1994).

2. MATERIALS AND METHODS

Study Area: the study was conducted in DNA Labs Kaduna, Northwest geopolitical zone of Nigeria. It is a facility that receives samples for HCV viral load and genotyping from all the seven states of northwest geopolitical zone (Kaduna, Kebbi, Kano, Katsina Zamfara, Jigawa and Sokoto) which makes it suitable for this research.

Subjects: the study comprised of 173 HCV antibody positive patients that requested for HCV viral load and genotype from any of the seven states in northwest geopolitical zone.

Inclusion and Exclusion Criteria: the inclusion criteria comprises of hepatitis C antibody positive patients of all sex and age. The study excludes patients outside northwest geopolitical zone or hepatitis C seronegative patients.

Specimen Collection: blood samples were collected by standard venopuncture without undue pressure either on arm or the syringe and dispensed in a plain container. It was allowed to clot for 30min and spun at 10,000 rpm for 5minutes in order to obtain serum. The separated sera were stored at -20°C until used for analysis.

Viral RNA Extraction: the extraction was conducted using Accuprep® viral RNA extraction kit. Binding buffer was used to lyse the cells in the serum followed by alcohol precipitation. The precipitated protein was filtered using a binding column, washed and eluted to obtain the RNA.

Master Mix and Viral Load Preparation: Accupower HCV-1111 (IVD) was used for the preparation of master mix. HCV lyophilized premix was reconstituted with ultra-pure water and eluted RNA was added. The mixture was taken immediately into a real time PCR machine (Roche) light cycler for the reverse transcription (cDNA) and viral load was ascertain upon completion of the run.

Genotyping: reconstituted RT-PCR premix was mixed with a specific volume of the eluted RNA and cDNA synthesis was performed at 50°C for 1 hour. Nested PCR was conducted using UTR1 and UTR2 primers for the 1st round, while UTR3 and UTR4 primers were used for the 2nd round, using the conditions (94°C for 1 minutes, 94°C 25 for seconds, 44°C for 40 seconds, 72°C for 1 minute and 72°C for 10 minutes) and (95°C for 1 minute 95°C for 20 seconds, 53°C for 30 seconds, 72°C for 25 seconds and 72°C for 10 minutes) respectively. The PCR product was ran on a 1.5% agarose gel at 120 volt, band size was compared with the molecular ladder by placing the gel on a UV light source and the right band was carefully cut for the gel extraction. Upon completion of the gel extraction, sequencing reaction was performed using the 2nd round reverse primer (UTR4) and the cycling was repeated 30 times. The sequencing reaction product was cleaned up and taken to the Beckman Coulter sequencing machine, obtaining the electrophorogram at the end of the run. The sequence was generated and BLASTED accordingly on GENE BANK to ascertain the HCV genotype.

3. RESULTS

Table 1: Percentage Distribution of the HCV Genotypes

Genotypes	No. of Patients	Percentage (%)
1b	93	54
1a	64	37
1c	2	1
2a	9	5
2b	5	3
TOTAL	173	100

Table 2:HCV Viral Loads with Respect to Genotypes

GENOTYPES	VIRAL LOAD (IU/ML)			
	<200,000	200,001 – 400,000	400,001 – 600,000	> 600,000
1b	27	11	39	16
1a	19	9	26	10
1c	0	2	0	0
2a	0	3	4	2
2b	2	0	2	1
TOTAL (Patients)	48	25	71	29

4. DISCUSSION

Hepatitis C viral disease plays a negative impact on the lives of many people globally, the distribution of its genotypes vary with respect to geographical location. Genotypes 1-3 being the worldwide distributed genotypes (Dusheiko *et al.*, 2005; Esfahani *et al.*, 2010).

The current study revealed that genotypes 1 and 2 were found to be the major causes of the disease in Northwestern part of Nigeria; with the subtype 1b having the highest number of patients (54%) and only (1%) have been found to be subtype 1c while 1a, 2a and 2b were found to have (37%), (05%) and (03%) respectively. A similar research by Jinget *et al.*, (2015) shows a predominant percentage of subtype 1b among the test subjects, which is in support of the present study. This finding contradict with the study conducted by Indian author Anita Charkravati *et al.*, (2011) and Pakistan author Suliman *et al.*, (2014) where genotype 3a was found to be the predominant subtype. It is however in contrast to the findings of Xie *et al.*, (2016) and Ederth *et al.*, (2016) that found 1a to be the subtype with highest percentage in USA and Sweden respectively. Findings by Joseph *et al.*, (2015) also found genotype 2 as the leading cause of HCV in Ghana accounting for about 80% of HCV cases.

The total of 48 patients with viral load < 200000 IU/ml was recorded, with the genotype 1b taking the highest number of patients, 27 (56.3%) while 1a has 19 (39.6%) number of patients and 2 (4.2%) patients represent 2b genotype. None of patients was found with the genotype 1c and 2a.

In the category of viral load of 200001 – 400000 IU/ml, the study revealed a 0% patient with HCV genotype 2b among the 25 patients within the range. Eleven (44%), 9 (36%), 2 (8%) and 3 (12%) patients were found to be infected with the viral genotype 1b, 1a, 1c and 2a respectively.

Out of the 71 HCV infected individuals within the range of 400001 – 600000 IU/ml viral load, 39 (54.9%) patients have 1b genotype, followed by 1a with 26 (36.6%) while 4 (5.6%) patients were found to have 2a and genotype 2b has 2 (2.8%) number of patients but there was no patient with 1c.

Among the 29 patients with viral load > 600000 IU/ml, genotype 1b has the highest prevalence of 16 (55.2%). There was no patient with HCV genotype 1c while 1a, 2a and 2b were found to have 10 (34.5%), 2 (6.7%) and 1 (3.4%) patients respectively.

5. CONCLUSION

In conclusion, the findings of the present study revealed that 93 (54%) patients were infected with genotype 1b, followed by 1a with 64 (39%), 1c, 2a and 2b with 2 (1%), 9 (5%) and 5 (3%) respectively. However, genotype 1b was found to have the highest prevalence, while 1c with lowest number of infected individuals. Moreover, in all the ranges of viral loads in the study, 1b genotype was found to have highest prevalence.

6. RECOMMENDATION

From the current study, it is recommended that the genotype and viral load of the virus should be known before commencement of therapy for any HCV positive patient and also to ensure effective management of the patients, viral load should be monitored accordingly.

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