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Sero-Prevalence and Circulating Genotypes of Hepatitis C Virus Among Diabetic Patients Attending Federal Medical Center, Keffi, Nasarawa State, North Central Nigeria

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ABSTRACT

Frequently, when people have hepatitis C virus infection, diabetes is often not taken into consideration. Surprisingly, these two conditions are much more closely linked than people think. The association in the hepatitis C and diabetes remains unclear and controversial in various publications. In Nigeria, the prevalence of hepatitis C virus in diabetic patients is not well documented. This study aimed is to determine prevalence of hepatitis C virus (HCV) and its circulating genotypes among diabetic patients attend the Federal Medical Center, Keffi, Nasarawa State, Nigeria. Three hundred and ninety-eight (398) blood samples were collected from consenting diabetic patients aged 24-64. These were 231 male patients and 167 female patients. A structured questionnaire was used to gather socio-demographic information from the patients. Blood samples from each participant were tested for HCV antibodies using a rapid test kit and enzyme-linked immunosorbent assay (ELISA). 7 samples tested positive in a rapid test, while 5 were confirmed positive using ELISA. All samples that tested positive for anti-HCV antibodies underwent genotyping using type-specific PCR primers. The prevalence of HCV infection in diabetic patients tested was (1.3%). In diabetis status, male subjects had a higher prevalence of (1.3%) compared to female subjects (1.2%). The gender differences were statistically insignificant (p>0.05). Additionally, the study found that HCV genotypes 2, 3 and 4 were circulating in the population, with genotypes 2 and 4 being more frequent. The major risk factors observed in the study included a history of surgery, blood transfusion, and sexually transmitted diseases. Giving these findings, it is recommended that a larger study be conducted to gather more epidemiological data on the prevalence of HCV among diabetic patients in Nigeria.

Keywords: *hepatitis C, Elisa, diabetic patients, questionnaire, genotyping*

1. Introduction

Hepatitis C virus, belongs to the family Flaviviridae, a single-stranded RNA virus with positive sense. It causes liver inflammation and it is estimated that over 58 million people worldwide are living with HCV. In developed countries, the prevalence of HCV is usually around 1 to 2%. [1] *Hepatitis C virus* (HCV) and diabetes are significant global health concerns of high morbidity and mortality rates, especially for the developing countries [2]. There is close association between hepatitis C virus and diabetes, with diabetes often reported as a complication of HCV infection [3]. Diabetes, especially mellitus, has been found to alter the course of hepatitis C even in the insulin resistance (IR) stage, which precedes the development of overt diabetes [2]. This is because the virus affects the liver, which plays a role in storing glucose. If the liver cannot function properly due to viral damage, it can result in high blood sugar levels and insulin resistance [4]. Hepatitis C is an infection that causes inflammation of the liver, it can either be an acute or chronic illness, that can be fatal [5]. The Hepatitis C virus is normally transmitted through contact with the blood of a person infected by the virus; this is generally possible via sharp objects, sexual intercourse, and childbirth [6]. Acute hepatitis c is a short-term illness lasting for about six months, after which, the body naturally (for some people) gets rid of it without them noticing, they are usually asymptomatic and do not usually lead to serious health problems, while chronic hepatitis c infection (for most people) up to 85% takes from six months and above, leading to lifelong phase, causing severe health issues like liver cancer and cirrhosis. There are many types of hepatitis c infection, known as genotypes. There exist seven genotypes and sixty-seven sub-types. Chronic hepatitis c takes the same pattern regardless of the type of genotype [7].

2. Materials and Methods

2.1 Study population

The study subjects includs both in- and out-diabetic mellitus patients of both genders from within Keffi town and the surrounding villages who were accessing medical care at Federal Medical Centre Keffi.

2.1.1 Study design
The study was cross-sectional study involve the both male and female diabetic adult patients. Each participant's consent was sought and socio-demographic and clinical information were obtained using a structured questionnaire.

2.1.2 Ethical consideration
Ethical clearance to conduct this study was sought from the Ethical Review Board/Ethical Committee of the Federal Medical Center (FMC) Keffi.

2.2 Sample collection
The chemical pathology unit of the laboratory was used as the collection point. A total of 398 blood samples were collected from consenting patients. The samples were collected by venipuncture.

2.3 Sample processing
To obtain serum, each blood sample was allowed to stand for 15-30 minutes and centrifuged at 3000 rpm (revolution per minute) for 10 minutes at 37°C. The sera were harvested and transferred into labeled cryovial tubes for storage. All samples were stored at minus 15°C in the Chemical Pathology unit freezer of FMC Keffi until ready for use.

2.4 Laboratory Analysis
2.4.1 Screening for Anti-HCV
The Biopanda HCV Rapid test kit(BiopandaDiagnostic, United Kingdom) was used, for the screening of HCV according to the manufacturer's instructions. It is a rapid test kit for the anti-HCV antibodies

2.4.2 Screening for Anti-HCV using ELISA
Qualitative detection of antibodies of hepatitis C virus in human serum, which is based on the Enzyme Linked Immunosorbent method was carried out using an anti-HCVELISA test kit (Qingdao Hightop Biotech Co., Ltd.) according to the manufacturer's instruction.

2.4.3 Test Procedure
The anti-HCV test reagents were allowed to equilibrate to room temperature for more than 15minutes. The wash buffer was diluted at the rate of 1:40 dilution by adding 5 µL of wash buffer to 5µL of distilled water. Labeled test serum samples from the diabetic patients corresponding were dropped into the wells in the 96 microtitre plate. Each microtitre plate of 96 wells had two negative control wells, a positive control well, and a blank control well. One hundred microlitres (µL) of buffer solution for HCV antibodies (sample diluents) was added in the corresponding wells in the microtiter plates (except in the blank well, negative control wells, and positive control wells).10µL of the serum samples were added in the corresponding well and then mixed thoroughly by using the pipette. Negative control (100µL) and positive control (100µL) were added to the negative control wells and positive control wells (except in the blank well) respectively. The plate was gently shaken to mix and incubated at 37^0 C for 60 minutes. At the end of the first incubation, the plate cover was removed and discarded. Tris-buffered saline with 0.1% Tween ® 20detergent (Wash buffer) was added to each well, and allowed to stay for 20 seconds before the content was tipped out and tapped on bloating paper to remove excess material in the wells, this washing procedure was repeated 5 times.

After washing cycle, microtiter plate was turned over onto blotting paper, tapped to the further remove the material. Fifty µL of Enzyme conjugate was added (except in the blank well) and the plates were properly sealed and incubated at 37^0 C for 30 minutes. Washing with wash buffer was done repeatedly 5 times. Chromogenic substrates A and B (50µL each) were added (except in the blank well) and then incubated at 37^0 C for 30 minutes. Fifty µL of Sulfuric acid (Stop Solution) was added to each well (except in the blank well). It was then gently shaken to mix and the optical density (OD) was read within 10 minutes.

2.4.4 Determination of HCV Genotypes by Nested PCR
Two-step nested reverse transcriptase polymerase chain reaction (RT-PCR) for HCV detection and genotyping [8-9] was used.

2.4.4.1 Polymerase Chain Reaction
Using the AccuPower Hot Start PCR PreMix PCR kit by Bioneer Inc. USA., the product of the first amplification reaction of the test samples was used as the template for the second PCR. During this second PCR, four PCR tubes were labelled and 16µl of PCR-graded water (DH_2O) was added into each tube and 2µl of the first round PCR product which served as the template, was added followed by the addition of 2µl of the second primer KY78 and KY80 (5'- GCAGAAAGCGTCTAGCCATGGCGT -3') and (5'CTCGCAAGCACCCTATCAGGCAGT -3') was added to each of the tubes containing the product of first PCR. This is to amplify a 244-nucleotide region of the 5′ untranslated conserved region (UTR) of the HCV genome. Thermo cycler (PTC – 100TM MJ – Research, INC, Peltier) conditions were set as follows; initial PCR activation steps for 5 minutes at 94°C, pre-denaturation for 30 seconds at 94°C; denaturation for 30 seconds at 60°C, annealing for 30 seconds at 72°C and extension for 30 seconds at 94°C (35 cycles were performed by going back to step two) and final extension for 5 minutes at 72°C.

2.4.4.2 Gel Electrophoresis and Visualization
The PCR products of the 3 reactions were confirmed on 2% agarose gel, visualized under UV light. The gel was prepared by weighing 2.2g of agarose (QD LE Agarose, Green BioResearch, USA) into 100mL of 1X Tris Acetate EDTA (TAE) buffer in a conical flask. The slurry was heated in a microwave for two minutes to properly dissolve the agarose. The solution was then allowed to cool to 60^{0}C. Ethidium bromide stain-intercalating agent (12µL) was added and mixed thoroughly by rocking. The casting of the molten gel was done by placing a comb on the mold and then gently pouring the molten agarose into the mold. The gel was allowed to solidify for 30 minutes after which the comb was removed to create wells for the molecular weight ladder and the amplified DNA samples. The tray together with the solidified gel placed in the electrophoresis tank. A 25bp DNA molecular weight marker of 6µL was loaded into the first wells to enable size estimation of the resolved bands. About 100 mL TBE buffer was then poured into the tank to cover the cast. Five microlitres of the PCR products (DNA) were then loaded slowly into their respective wells in the submerged gel. The electromotive force was applied to the gel and was run under a constant voltage of 120 for the 30 minutes. After 30 minutes, gel was removed from gel-box, an excess buffer from surface of gel was drained off and gel tray was placed on the paper towels to absorb extra running buffer. The separated DNA fragments in gel were visualized by illumination with UV light and the pictures were taken with gel documentation system

(Gel Doc 2000, BIORAD, USA) and to determine 244bp of the untranslated conserved-region.

2.5 Statistical Analysis

The ata entry and statistical analysis were performed with (SPSS) (SPSS, Inc., Chicago, IL). The descriptive data were presented as simple summaries in tables, frequencies, and bar charts. The chi-square test was used where appropriate to establish statistically significant differences between participants, variables and prevalence rates. Probability values (p-values) ≤ 0.05 were considered significant.

3. Results

The study participants comprised 231 males (58.0%) and 167 females (42.0%) with male. The ages of participants ranged between 24-79 years, more than half 65.3% of them were married. Regarding educational status, most of them 45.5% had secondary education. About half of the participants 50.3% were farmers, 18.3% were civil servants. 15.1% were businessmen and women, 3.8%were housewives, 2.5% in other occupations (Table 1).

Clinical history revealed that 20.6% of them have had a blood transfusion, 26.4% had STD, 13.6% had tattooed and 13.6% had surgery. When stratified based on social factors, 223 patients representing 56.0% had single sexual partners while 24.6%, 13.8%,and 5.5%had 2, 3, and multiple sexual partners respectively. Only 1.0% of the participants smoke. About 51.5% of them residein rural areas, 34.9% in urban areas; and 13.6% in other settlements (Table 2).

The 398 participants screened for HCV using RDT and ELISA, eight and five representing a prevalence of 2.0% and 1.3% respectively were positive (Figure 1). In relation to gender, 1.3% of the males tested positive while 1.2% of the females were found to be positive, there was no significant association between gender and prevalence of HCV in this study (p>0.05) (Table 3).

Table 1: Demographic Characteristics of Participants

Variable	Number of participants (n=398)	Percentage (%)
Gender		
Male	231	58.0
Female	167	42.0
Age (Years)		
24-33	69	17.3
34-43	54	13.6
44-53	92	23.1
54-63	70	17.6
≥64	113	28.4
Marital Status		
Married	260	65.3
Single	138	34.7
Level of Education		
Non-Formal	84	21.1
Primary	31	7.8
Secondary	181	45.5
Tertiary	102	25.6
Occupation		
Business	60	15.1
Civil Servant	73	18.3
Farming	200	50.3
Others	65	16.3

Table 2: Clinical and Social Characteristics of Participants

Variable	Number of Participants (n= 398)	Percentage (%)
Blood Transfusion		
Yes	82	20.6
No	316	79.4
History of STD		
Yes	105	26.4
No	293	73.6
Scarification mark		
Yes	54	13.6
No	344	86.4
History of Surgery		
Yes	54	13.6
No	344	86.4
Multiple sexual partners		
Yes	223	56.0
No	175	44.0
Smoking		
Yes	4	1.0
No	394	99.0

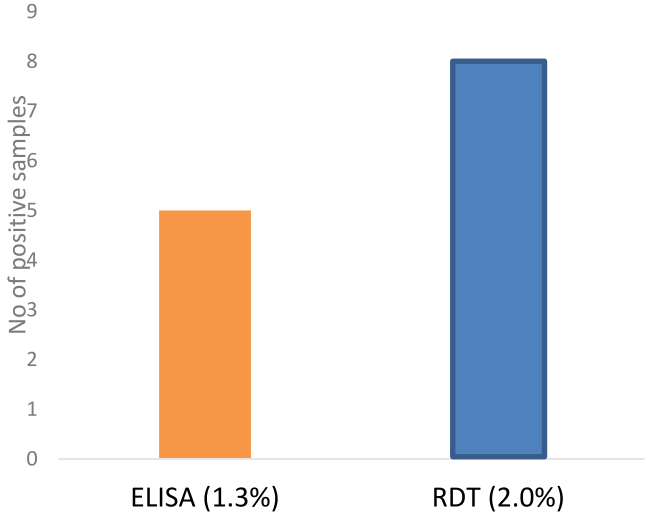

Table 3: Relationship between prevalence of HCV and demographic characteristics of diabetic patients attending Federal Medical Centre Keffi

Variable	No. screened (n=398)	No positive (%) (n=5)	P-value
Gender			
Male	231	3(1.3)	0.999
Female	167	2(1.2)	
Age (Years)			
24-33	69	2(2.9)	
34-43	54	2(3.7)	
44-53	92	0(0.0)	0.127
54-63	70	0(0.0)	
≥64	113	1(0.9)	
Education			
Non-Formal	84	4(4.8)	
Primary	31	0(0.0)	0.014*
Secondary	181	0(0.0)	
Tertiary	102	1(1.0)	
Occupation			
Business	60	1(1.7)	
Civil Servant	73	1(1.4)	
Farming	200	2(1.0)	0.204
Others	65	1(0.0)	

Statistically significant

An examination of the age distribution of diabetic patients, as presented in Table 3, showed that out of the five age groups categories investigated, participants aged 44-53 and 54- 63 years were all negative for Hepatitis C. However, 2(2.9%) of the patients in the age group 24-33 years,2(3.7%)in the age group 34 – 43 years, and 1(0.9%) in the group age ≥ 64respectively were positive for Hepatitis C.

With the participants' educational status, particularly those with primary and secondary education, the sample showed that all tested negative for the virus. However, 4(4.8%) participants who had non-formal education were positive for it, and it was the highest recorded HCV infection. All categories of workers had at least one infection each (Table 3), to identify high-risk groups and understand the epidemiology of HCV infection within the diabetic patient population, participants were screened based on clinical risk factors. Results from Table 4 showed that patients with a history of blood transfusion reported a prevalence of 1.2%, patients with a history of surgery recorded a prevalence of 7.4%, patients with a history of sexually transmitted disease reported a prevalence of 3.8%, and those with tattoos recorded 1.9%, positivity for HCV infections, subjects were examined to understand the distribution and complex interplay between social lifestyle factors and HCV infection in the context of diabetes patients. Results from Table 5 showed that subjects with a history of smoking recorded 25.0% positivity, and those with multiple sexual partners had 2.2% positivity to HCV infection. The relationship between number of sexual partners (P= 0.001) and smoking (P = 0.049) were risk factors for HCV infection. Agarose gel electrophoresis shows different HCV genotypes 2, 4, and 3 respectively. (Plates 1 and 2).

Table 4: Relationship between prevalence of HCV, Clinical factors, and Social Factors of diabetic patients attending Federal Centre Keffi

Variables	Number examined (n=398)	Number-Positive (%) (n=5)	P-value
Blood Transfusion			
No	316	4(1.2)	0.007*
Yes	82	1(1.2)	
History of STD			
No	293	1(0.3)	0.018*
Yes	105	4(3.8)	
Piercing/Tattoo			
No	344	4(1.2)	0.520
Yes	54	1(1.9)	
History of Surgery			
No	344	1(0.3)	0.001*
Yes	54	4(7.4)	
Multiple Sexual Partner			
Yes	223	5(2.2)	0.001*
No	175	0(0.0)	
Smoking			
Yes	4	1(25.0)	0.049
No	394	4(1.0)	
Settlement			
Rural	205	1(0.5)	0.175
Urban	139	4(2.9)	
Others	54	0(0.0)	

Statistically significant

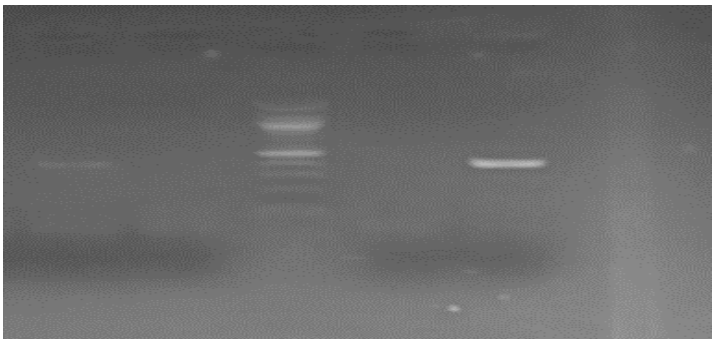

Plate 1: Agarose gel electrophoresis showing different HCV genotypes. Lane 1 and 4 showed genotype 4 (229bp) While lane L represents the molecular ladder.

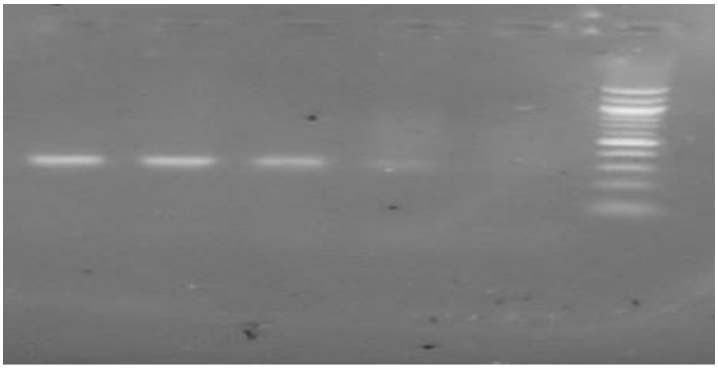

Plate 2: Agarose gel electrophoresis showing different HCV genotypes. Lane 1, 2 and 3 showed genotype 3 (200bp) While lane L represents the molecular ladder.

4. Discussion

The participants were first screened the HCV antibodies with the help of rapid diagnostic strips followed by ELISA. Seropositive cases were genotyped by polymerase chain reaction and gel electrophoresis. Of the 398 participants, 2.0% were positive for using rapid diagnostic test strips while 1.3% was seropositive using the ELISA method.Three previously positive samplesusing RDT were found to be negative using the ELISA technique. This suggests that ELISA is the more sensitive technique; this is a serious source of concern as the majority of labs use only the rapid technique for diagnoses.

The seroprevalence of hepatitis C in the current study was 1.3%, similar to studies reported in Enugu and Ibadan Nigeria with a prevalence of 1.0% [10] and 0.9% [11]. It is however lower when compared to the work of [12] in Enugu where the HCV occurrence rate among diabetic patients was found to be 14.4%. [13] reporteda higher prevalence of 3.0% among blood donors in Masaka Nasarawa State. [14] also reported 24.2% among diabetic patients attending the University of Ibadan Teaching Hospital. However, [15] reported a lower prevalence of 0.7% among diabetic patients in Lagos. Studies in other parts of the world like that of [9] and [16] reported a high prevalence of 8.0% and 5.7% in Asia respectively. Differences in the prevalence rate of HCV obtained from various regions globally depict geographical diversity, which can be ascribed to exposure to various risk factors thatcan enhance the spread and transmission of this virus among individuals. Similarly, it could also be a result of the different sensitivities of the test kits used for screening participants. The prevalence rate of HCV infection among males was 1.3% while the female gender had 1.2% sero-positivity.

This finding is in line with the result [11] where the prevalence of anti-HCV among diabetic-males was close to that of the females. A similar occurrence in both sexes shows that gendermay not be an attributed risk factor for HCV infection in diabetic patients. The prevalence of HCV infection (3.7%) observed amongst subjects aged 34-43 years, was the highest in this study. This finding follows that of [10] where the seroprevalence of HCV increasedin older participants and it was significantly higher in the group aged 31-40 years which is almost similar to the results obtained [17] showed a higher prevalence rate among patients aged 35-44 years thereby concurring with the result obtained in this study. The high seropositive observed in the older age group could be attributed to possible differences in social practices, decrease in physical mobility, decline in immune resistance to infections, and reduced rate of medical examination compared to younger age groups.

The educational background of the subjects screened showed that 4.8% of individualswith non-formal education are positive for the virus infection. This is similar to the work [18] which recorded 4.6% prevalence. The reason for this could be as a result of non-adherence to preventive measures. It could also be because they are ignorant of the availability of materials that propagate knowledge about prevention and control. Similarly, participants with multiple sexual partners as well as those with a history of STD had the highest seropositive index, since HCV can be sexually transmitted it is not surprising that prevalence is higher among those with a history of STD and multiple sexual partners. This is similar to an earlier report of a study [19], who reported people with multiple sexual partners as being a high-risk group of infection by HCV. The viral infection is known also to be sexually transmitted so multiple partners will naturally increase the probability of getting infected. The HCV being a blood-borne virus, the seroprevalence among individuals who had undergone blood transfusion was not statistically significant and this agrees with the work [20]. Among subjects that had a history of blood transfusion in this study, only 1.2% was found to be positive for HCV infection, it is known that blood and blood products are potential sources of transmission for HCV infection [21]. This may also suggest that transfused blood is usually properly screened for certain blood-transmissible viruses before use. The national guideline for the prevention care and treatment of viral hepatitis in Nigeria [22], requires a confirmatory test with HCV RNA after a positive antibody serology before the commencement of treatment. It could also be a reflection of the relatively low prevalence of the virus in eligible blood donors. A high rate of HCV infection was equally observed in participantswitha history of surgery, tattoo/facial, and marks. Prevalence rates ranged from 3.8%, 1.9%, and 7.4% respectively,this finding was in contrast to an earlier report [21] which shows that risk factors for HCV were obscure in Nigeria, although that study conducted in another geographical zone in Nigeria. The results of this study revealed the presence of genotypes 2,3 and 4. This agrees with the review study by [8] which shows that all the major genotypes except genotype 6 are present in Africa. They also showed thepresence of mixed genotypes in Africa. Until recently, it was not necessary to determine the genotype of the infecting virus before the commencement of treatment, Accurate HCV genotyping is important for predicting the response to antiviral therapy since genotypes 1 and 4 respond poorly to interferon than genotypes 2 and 3 [23,24,25].

5. Conclusion

This study aims to determine the seroprevalence of hepatitis C virus infection among diabetic patients at Federal Medical Centre Keffi, Nasarawa State. This current studies recorded an overall seroprevalence of HCV among diabetic patients at FMC to be 1.3%, 1.3 may not be statistically significant as reported, compared to the number of samples collected at the beginning of this study, but also, confirms that diabetes can be caused by hepatitis C virus and, no matter how insignificant the percentage may be statistically, it is still human lives we are talking about, therefore, there is cause for alarm as HCV is never considered when a patient is being confirmed to be diabetic. This is possible because the hepatitis C virus is known to infect the liver, the liver is also responsible for the storage of excess glucose (blood sugar) in the blood for later, once the liver is impaired or damaged due to this viral infection, it may not be able to carry out its normal function, thereby, leading to the high sugar level in the blood, these studies confirm that patients who tested positive for HCV are often ignorant of the fact that they need to go for further tests (ELISA and Molecular) to confirm the earlier result and the genotypes responsible for the infection for proper treatment, the statistically significant risk factor in this study shows that HCV infection can be prevented if the general public is cautious of the dangers and causes of HCV infection.

6. Conflict of Interest statement

The authors declare no conflicts of interest

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