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Assessment of Clinical Chemistry Parameters and Viral Load of Hepatitis C Virus among Patients Infected with Hepatitis B Virus Attending Alex Ekwueme Federal Teaching Hospital (AE-FETHA), Abakaliki, Ebonyi State, Nigeria

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Abstract

Hepatitis A and B are the most common causes of chronic hepatitis infections globally. This study aims at evaluating the clinical chemistry parameters of Hepatitis C virus among individuals infected with Hepatitis B virus attending Federal Teaching Hospital (FETHA), Abakaliki, Ebonyi State, Nigeria. Data such as demographic, laboratory and clinical data were collected from the hospital. Blood samples were also collected from 480 consented individuals for the screening and the presence of HBsAg was detected. Some chemistry analyses were performed to unveil the level of their immune responses using laboratory standard procedures. Of a total 480 consented patients screened, 101 individuals were reactive to hepatitis B virus surface antigen. Data obtained in our study showed an overall percentage prevalence of 45 (male patients, 24.8, and female patients 75.2). In analysis of HCV-IgM, the higher percentage was noticed among the male individuals infected with hepatitis B virus (20 %) and female subjects with 13.2 %. The IgG was higher among male subjects with 16 % and female patients with 14.5 % while patients of 52-62 years of ages had high percentage of 50 each for both IgM and IgG. Occupation was also considered as a demographic factor and farmers presented higher percentage among the occupations observed, there was a significant difference between the parameters observed in respect to their occupations ($p < 0.05$). Results of chemical analyses of patients positive for HCV showed higher viral load among the male counterparts and alanine aminotransferase level is higher for patients between the ages of 19-29 years. Meanwhile, increase in creatinine level was noticed among the businessmen. Conclusively, hepatitis B and C viruses are global challenges and require immediate attention.

Keywords: Hepatitis B Virus, Hepatitis C Virus, Clinical Chemistry, IgM, IgG, $CD4^+$, Viral Load
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Introduction

Hepatitis B and C viruses are viral infections that attack the organ such as the liver and therefore resulting to both acute and chronic diseases (Bullock *et al.*, 2002). The incubation period of both viruses is 2-6 months, (60-180 days) between 15-29 years. Both viruses can result to acute and chronic stages of hepatitis, starting from severity to a mild illness which lasting for a few weeks then to a serious lifelong illness (Idoko *et al.*, 2007).

About 250 million individuals are currently prone to viral hepatitis chronically, especially hepatitis B viruses (Surface antigen positive for hepatitis B at minimum of 6 months and hepatitis C virus globally (WHO, 2015). In some sub-Saharan Africa, about 25.4 million are now infected with this virus and 13.3 million in Nigeria (Thio *et al.*, 2009). The

prevalence of two viruses in Nigeria has been reported as 17.1 % among female sex workers (Ajuwon *et al*., 2016).

Occurrence of symptoms take place within four to six weeks after individuals being infected which may last from one or two weeks to several months (Abdel-Hamid *et al.,* 2002).This signs and symptoms can include yellowing of the skin, whitening of the eyes, and under the fingernails (jaundice), dark urine or pale stool, tiredness, fatigue, fever, abdominal pain, loss of appetite, nausea, diarrhea, joint pain, vomiting and general body weakness (Cheesbrough, 2006).

Both HBV and HCV share common mode of transmission, therefore, living together in the same host at significantly high rates (Idoko *et al*., 2007). Vertical and sexual transmission is very low, but transmission is predominantly by blood contact.

The commonly used method of diagnosis of HBV and HCV viruses is antibody testing. Enzyme Linked Immunosorbent Assay (ELISA) method is useful especially during the infants testing from mothers that are positive for HCV, i.e., elevation of serum alanine transferase, serum aspartate transaminase, and bilirubin. Serology tests can also be used to detect the presence of HBV in a serum and the serological diagnosis is based on the detection of HBsAg, HBeAg, anti HBe, anti-HBs and anti- HBc and molecular analysis (Bartenschlager *et al*., 2003).

Treatment with antiviral medication is recommended. Lamivudine has been extensively used in the treatment of HBV among pregnant woman in attempt to prevent prenatal transmission of hepatitis B virus infection with mixed success. Individuals with more complications should undergo treatment first because the risk of complications is determined by the degree of liver scarring (Thio *et al*., 2009).

Materials and Methods

Sample collection: About 5ml of blood samples were collected each from a total of 101 HBV positive patients visiting FETHA for a period of one year (2017-2018). Serum samples were separated by low-speed centrifugation at 500 rpm for 5 minutes. Then, serum was transferred into labeled sterile cryovials and stored at -20^{o}C in the sterile container. The stored sample was transported to Nigerian Institute of Medical Research (NIMR), 6 Edmond Crescent, Yaba, Lagos for the analysis.

Serology Test for HCV IgM (ELISA Method)

A 200 µl of HCV- IgM was added to the negative control in triplicate (B, C and D wells) containing the antigen coated well, the blank (A well) was left without adding any diluents and it was incubated for 1 hour. A 200 µl of HCV- IgM antibodies was added to the calibrator control (E and F wells) containing the antigen coated well and 200 µl of HCV-IgM was also added to the positive control (G wells) containing the antigen coated well.

A 200 µl of the sample diluents (DILSPE) was added to all the well starting from H except the controls. The colour was now observed from light green to dark bluish green. Therefore, 10 µl of the sample (plasma) was then added to all well except the controls and the blank. A50 µl of the DILAS was dispensed to all the wells including the control, calibrator except the blank and the colour turns to dark blue. It was then covered with adhesive sealing foil and the microplate was incubated for 45mins at 37^{o}C. After incubation, the microplate was washed for 4-5 cycles using an automated washer machine, it was empty, and the residual liquid was tapped out.

A 100 µl of Enzyme conjugate was pipetted into each well except the blank and it was covered with the sealer and the microplate was then incubated again for 45 min at 37^{o}C. After the 2nd incubation, the microplate was then washed for 4-5 cycles.

Serology Test for HCV-IgG (ELISA Method)

Well A1 and B1 wereleft empty as blank. A 100 µl of HCV- IgG was added to the calibrators in duplicate, another 100 µl HCV-IgG was also dispensed in dissolved control serum in duplicate to the same volume of 100 µl of diluted samples. The control serum was used to verify that the whole analytical system works as expected. The microplate was incubated for 60mins at 37 ^{o}C and it was washed using an automated strip washer ELx50 for 4-5 cycles. In all the wells except A1+B1, 100 µl of enzyme conjugate was dispensed and the microplate was incubated for 60 min at 37^{o}C at the end of 2nd incubation, the microwells was then washed for 4-5 cycles and a 100 µl chromogen/ substrate was pipetted into all the wells, A1+B1 included.

The microplate was protected from light by incubating it at a room temperature in a dark room usually (18-24^{o}C) for 20 min. Wells dispensed with positive samples, the control serum and the positive calibrators was turn from clear to blue color. At this stage, a 100 µl of Sulphuric acid was dispensed to all the wells including A1+B1 to block the enzymatic reaction. Addition of the stop solution turns the positive samples and control from blue to yellow and the color intensity of the solution in each well was measured using molecular automated Devices {(E-Max Elisa reader at 450nm filter) (Optical Density).

HCV- RNA Viral Load Test

This was done using an RNA Polymerase Chain Reaction (PCR). The plasma sample which is the best choice for viral load was used atCentre for Human Virology and Genomics (CHVG), Nigerian Institute of Medical Research, Yaba, Lagos. The samples and control were pipetted into their corresponding tubes with 3 controls for HCV-RNA assays which are negative, low positive and high positive controls. Barcoded chips were fixed into the sample racks containing the controls and the samples, both the controls and samples were placed into the racks. The sample was labelled properly, and the identification number was given to each sample. Reagent was brought to room temperature and PCR assay conducted.

Clinical Chemistry

Liver Function Test (LFT) and Creatinine

Procedure

The machine was switched on and it was allowed to initialize for about 5 mins. The 2 controls were used which are Precinorm (Normal controls) and Precipath (Pathological controls) and the values of the controls were plotted on LJ chart, and it was accepted or rejected based on Westguard rules which are based on the performances of the control's values in the acceptance range, the patient's samples were then assayed using the equipment (Chemistry auto-analyzer, Cobas C-311). The Samples were aliquoted into sample cups, and it was placed in the groove for the samples to run, the sample ID of the patients and the corresponding test was inputted into the computer connected to the chemistry auto-analyzer machine and the command was then run and the tests was assayed.

Results

Table 1: Positive HBV Patients Attending Federal Teaching Hospital Abakaliki (FETHA)

Sex	Total HBV Screened	HBV Positive Patients	%HBV positive Patients
Male	100	25	25
Female	380	76	20
Total	**480**	**101**	**45**

The results of individuals screened for Hepatitis B virus attending Federal Teaching Hospital, Abakaliki (FETHA) showed that, out of the 480 individuals screened, 101 (45 %) were positive. There were 25 males (25 %), and 76 females (20 %) a total of 48 % for both the male and females shown in Table 1.

Table 2: Clinical Chemistry Results of HCV-IgM and IgG of Infected Individuals Obtained from FETHA with Respect to their Sex

Sex	Total	% HCV-IgM& IgGPos	ALT	CRET	Viral load
		IgM			
Male	25	05 (20)	20.2	79	5,132
Female	76	10(13.2)	20.6	75	3.883
Total	**101**	**15(33.2)**	**40.8**	**154**	**9,015**

		IgG			
Male	25	04 (16)	23.3	80	5,245
Female	76	11(14.5)	20.6	75	3.247
Total	**101**	**15(30.5)**	**43.9**	**155**	**8,492**

The results of IgM screening showed that out of 25 males and 76 for females, 5(20 %) and 10(13.2 %) were positive for IgM, while for the IgG, 4(16 %) and 11(14.5 %) respectively were positive. Clinical chemistry parameters were higher in IgG than IgM, whereas IgM is higher in terms of their viral load than IgG .

Table 3: Clinical Chemistry Parameters of Individuals Positive for HCV-IgM and IgG with Respect to their Age in FETHA

Age	Total	%HCV-IgM and IgGPos	ALT	CRET	Viral oad
		IgM			
8-18	13	01 (7.7)	11.6	62	1,028
19-29	45	6(13.3)	15.0	79	3,670
30-40	28	3 (10.7)	20.0	75	4,571
41-51	08	-	-	-	-
52-62	06	03 (50)	24.4	80	6,535
63-73	01	-	-	-	-
Total	**101**	**13(81.7)**	**76.6**	**296**	**15,804**
		IgG			
8-18	13	01 (7.7)	11.6	62	1,028
19.29	45	8(17.8)	21.3	76	3,572
30-40	28	3(10.7)	19.4	79	3,461
41-51	08	-	-	-	-
52-62	06	03 (50)	24.4	79	5,572
63-73	01	-	-	-	-
Total	**101**	**15 (100)**	**76.7**	**296**	**13633**

The results of the HCV-IgM showed that age between 8-18 years had 1(7.7 %), 19-29, 6 were positive with of 40%, 30-40, 3(20 %), 41-51, 63-73 had zero percentand 52-62 had (33.3%). However, for HCV-IgG, age between 52-62 years gave 50 %, 8-18 1(7.7 %), 19-29 presented 8(17.8), 30-40 had 3(10.7), 41-51, 63-73 had zero percent and 52-62 presented 3(20) (Table 3).

Table 4: Occupational Distribution of HCV-IgM with their Clinical Chemistry and their viral load among HBV Infected Individuals in FETHA

Occupation	Total	%HCV-IgM and IgGPos	ALT	CRET	Viral load
		IgM			
Trader	10	01(10)	14.9	83	1,775
Teacher	07	-	-	-	-
Student	37	06(16.2)	16.9	76	3,379
Nurse	01	-	-	-	-
Farmer	09	03(33.3)	19.0	72	5,101
Bus.Women	27	4(14.8)	28.0	77	5,210
Hairdresser	01	-	-	-	-
Bus man	06	01(16.7)	29.6	96	9,422
Apprentice	01	-	-	-	-
Tailoring	02	-	-	-	-
Total	**101**	**15(91)**	**108.4**	**404**	**24,887**

		IgG			
Trader	10	01 (10)	14.9	83	1,775
Teacher	07	01(14.3)	32.1	61	6,857
Student	37	07(18.9)	16.9	75	3,055
Nurse	01	-	-	-	-
Farmer	09	02(22.2)	20.7	68	3,751
Bus.Women	27	03(11.1)	25.8	82	3,254
Hairdresser	01	-	-	-	-
Bus, men	06	01(16.7)	29.6	96	9,422
Apprentice	01	-	-	-	-
Tailoring	02	-	-	-	-
Total	**101**	**15 (100)**	**140**	**465**	**28,114**

The results for the IgM showed that traders had 1(10 %) for IgM, teachers, zero (0%) percent, students 6(40), farmers 3(20), businesswomen 4(26.7).

In respect to the IgG, farmers had 2(13.3 %), traders 10, teachers 1(14.3), students 7(18.9). The total percentage of their clinical chemistry and their viral load was higher in IgG than IgM (Table 4).

Table 5: Clinical Chemistry Parameter of Individuals Positive for HCV-IgM and IgG in Respect to their Educational Level

Educational Level	Total	%HCV-IgM& IgGPos	ALT	CRET	Viral load
		IgM			
No Formal Ed	15	2(13.3)	23.7	82	6,016
Pry. Sch.	22	5(22.7)	21.2	79	5,062
Sec. Sch.	31	03(9.7)	13.4	70	3,422
Tertiary Edu.	33	5(15.2)	24.3	77	3,378
Total	**101**	**15(60.9)**	**82.6**	**308**	**17,878**
		IgG			
NoFormal Edu	15	01(6.7)	14.3	89	4,571
Pry. Sch.	22	03(13.6)	19.0	85	4,393
Sec. sch.	31	04(12.9)	17.9	71	3,524
Tertiary Edu	33	07(21.2)	22.7	74	3,551
Total	**101**	**15(54.4)**	**73.9**	**319**	**16,039**

The result of the IgM revealed that primary and tertiary education certificate holders gave a percentage prevalence of 5(22.7) and 5(15.2) respectively,individuals without formal education and secondary school had 2(13.3) and 3(9.7) respectively (Table 5).

Discussion.

This study provides a comprehensive sentinel surveillance of HBV and HCV among individuals infected with hepatitis B virus visiting Federal Teaching Hospital, Abakaliki, Ebonyi State. Out of 480 screened patients, 101 were positive for HBV and blood samples were collected from the positive individuals in the hospital, females examined were 380 where 76 were positive (20%) and the male subjects were 100 patients where 25 of them were positive 25 %. This is not in line with the work done by (Craxi *et al.,* 2003 and Davis *et al*., 2010), who reported 55 and52 % positive cases of HBV in University of Nigeria Teaching Hospital, Enugu, and Enugu State University Teaching Hospital, (ESUTH) respectively.

The total percentage prevalence of 45 recorded among the male and female subjects

is in line with the work done by Taletela *et al*. (2008) who recorded a total percentage prevalence of 50 among male and female patients visiting Muhimbili National Hospital, California (Table 2).

The age group of infected patients with HCV-IgM was also evaluated. High percentage prevalence of 50 was observed among 52-62 years, out of 6 examine, 3 were positive. This was in accordance with the study conducted by Sanchez *et al.* (2004), who reported a high percentage of 53.3% for HBV IgM and 64.6 % for IgG between the ages of 50-60 years in Tanzania.

The Occupational Characteristics of a positive HCV-IgM was considered and our study revealed that farmers presented high percentage values of 33.3 out of 9 individuals examined.The results obtained from our findings was not in support of HBV prevalence studies conducted in Ibadan, western Nigeria by Adewole *et al.,* (2006), Ahmed *et al.,* (2005) which gave a percentage prevalence of 24.1 and 23.3 % respectively among students in University of Ibadan. The statistical analysis showed no significant difference among the variables in respect to the occupation ($p < 0.05$) (Table 4).

The occupation of individuals positive for HCV-IgG showed high percentage among the farmers (33.3), out of 9 examined, 3 were positive with the ALT (16.9 µL) CRET (76 mg/dL) and viral load (3,379 mg/L). There was no significant difference between the values obtained for the viral load of HBV infected individuals positive for HCV-IgG with respect to their demographical data ($p < 0.05$). This was in consonant with the work done by (Davis *et al*., 2010) who reported an ALT level of 17.1µL among those undergoing dialysis at University of Maiduguri Teaching Hospital.

Based on the educational qualification of the individual's patients, our findings here revealed that primary school certificate holders presented a high percentage prevalence of 22.7. This was not in line with the work done by Imade *et al*. (2003) who reported a high percentage prevalence of 45.2 among students of Nnamdi Azikwe University, Akwa, Anambra state. The ALT, CRET and viral load for the primary education were 19 µL, 85 mg/dL and 4,393 mg/L respectively and tertiary education had 22.7 µL for ALT, 74 mg/dL for CRET and 3,551 mg/L for viral load (Table 6).

The results of the clinical chemistry and the viral load for positive HCV-IgG in respect to their sexes showed a total ALT, CRET and viral load of 19.0 µL, 79 mg/dL and 5,132 mg/L respectively. The 19.2, 77and 6,021 and 19.3, 67 and 5,221 reported by Adewole *et al*. (2006), among the female and male patients respectively agreed with our findings. When this was subjected to statistical analysis, there was no significant difference between the male and the female with respect to their ALT level ($p > 0.05$).

According to age groups,the results revealed that individuals between 52-62 years presented the high level of HCV-IgG like that of IgM with the percentage prevalence of 50 out of 6 consented, 3 were positive with the ALT (21 µL) CRET (76 mg/dL) and viral load (3,572 mg/L). The 50 % recorded in our study among the age groups of 52-62 years agreed with the study conducted by Ajuwon *et al*. (2016), who reported 57.3 % between the ages of 50-60 years.

Based on their occupations, our findings revealed that farmers gave the high percentage prevalence of 22.2 while 20.7 µL for ALT, CRET, 68 mg/dL and viral load 3,751 mg/L) out of 9 positive HBV subjects examined, 2 were also positive HCV-IgG.

Our findings based on the educational qualification of individuals showed that tertiary certificate holder gave high percentage prevalence of 21.2 out of 33 positive patients screened for HBV for the study, 7 were positive for HCV-IgG, Taletela *et al*. (2008), reported the same values.

The screening for Hepatitis B Virus among patients attending Federal Teaching Hospital, Abakaliki showed an overall percentage prevalence of 45 among infected individuals where higher percentage were recorded among male subjects (25 %) while female subjects presented (20 %) in our study. The results of the hepatitis C Virus Immunoglobulin, more prevalent rate was observed among the male patients infected with hepatitis B Virus (20 %) and female subjects with 13.2 %. The IgG were also found to be higher among the male counterparts with 16 % to female counterparts 14.5 % meanwhile, from 52-62 years of age revealed greater percentage of 50 each for both IgM and IgG (Dienstag *et al.,* 2007). It was noticed that farmers also gave high percentage among the occupation observed in our findings. Therefore, a

significant difference was noticedbetween the parameter observed in respect to their occupation ($p < 0.05$).
The level of education of an individual's results revealed that primary school certificate holder showed higher percentage whereas higher percentage of ALT were found among the farmers, this signified the level of liver damage (Gay *et al.*, 2001). This research provided opportunity to create awareness especially on hepatitis B virus where majority leaving in the rural area were not informed. Therefore, government should structure some health policies that will involve both HIV and HBV as a routine screening for antenatal patients visiting health care facilities in Nigeria. Both the acute and chronic states of hepatitis B and C treatments must begin immediately after positive diagnosis.

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