The immunological evaluate of Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) co-infection with plasmodium in Wukari, Taraba State, North East, Nigeria

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Abstract
Blood serves as a vehicle for transmission of blood-borne pathogens including hepatitis viruses and hemoparasities. In Taraba State, screening of blood for blood-borne pathogens does not fulfill the standard protocols and screening for malaria parasites is not practiced. This study was to determine the immunological evaluation of Hepatitis B Virus, Hepatitis C Virus and Plasmodium co-infection profile among individuals in Wukari. One hundred (100) venous blood samples was obtained from individuals age between 15 to 74 years randomly using a dry sterile syringe and needle; the blood is withdrawn from a suitable vein in the arm and the blood sample was slowly ejected into sample tube (EDTA container) and standard microbiological techniques were observed. The result denotes an overall prevalence of HBV, HCV and malaria parasites to be 15%, 6.0% and 2% respectively. It also shows in terms of sex and age groups that HBV has the highest prevalence within age group of 25-34, 4 (4.0%) for male and 25-34, 4 (4.0%) for female, while, HCV has the least prevalence within the age group of 15-24, 1 (10%) and 25-34, 1 (10%), for male and age group of 15-24, 1 (10%), 25-34, 1 (10%) and 35-44, 1 (10%) for females. The male population was the only one tested positive for malaria parasite from the age group of 15-24, 2 (2.0%). There was no co-infection of Hepatitis B virus, Hepatitis C and malaria, despite having prevalence of 15%, 6% and 2% respectively. The research on its own has shown that in order to reduce HBV, HCV and plasmodium co-infection, mass immunization of adults and antiviral drugs should be provided for those that are infected, while HBV, HCV and plasmodium co-infections screening programs should be instituted in all levels of institutions, communities and societies in the North East region, Taraba state in particular and the country in general to reduce the prevalence rate and level of transmission of hepatitis virus. This study also has added to the puddle of knowledge in this area of research.

Keywords: Co-infection, Hepatitis B Virus, Hepatitis C Virus, Plasmodium, Wukari, North East

1. Introduction
Malaria remains a major health threat worldwide. Endemic regions for malaria are also endemic for other infectious diseases that might affect the malaria infection [1]. Examples for such a common endemic infection sharing the same territory with malaria are hepatitis B virus (HBV) and hepatitis C (HCV) [2]. HBV stimulates a potent pro-inflammatory Type 1 immune response (Th1), which is of paramount importance for Plasmodium clearance; however, it is also incriminated in disease severity [3]. Whilst challenging, data on the effects of HBV on the clinical presentation of malaria are scarce [3]. Demonstrated in a mice model that intra hepatic HBV replication is inhibited by P. yoelii infection, moreover, production of interferon (IFN)-c and IFN-a/b is increased in the live [3]. In humans, information from a small study proposes that acute P. falciparum malaria alters HBV viremia in patients with chronic HBV infection [1].

Moreover, a study carried in Vietnam illustrated that patients with cerebral malaria had a slightly greater vulnerability to demonstrate HBV surface antigen (HBsAg) sero-positivity [1]; nevertheless, the aforementioned study failed to show any significant relation between the overall risk of death caused by severe falciparum malaria and positivity for HBs Ag [3]. However, there is lack of strong evidence supporting the suggestion that the clinical status of underlying hepatitis B-related liver disease is affected during malaria infection [2]. Furthermore, having the three infections sharing an intra-hepatic stage as part of their life cycles, interactions between the three pathogens have been proposed to occur at both immunological and cellular levels, not only this but on looking at their epidemiological maps, a clear intersection is seen between the areas of endemicity of the three pathogens [3]. Such interactions between HBV and malaria have already been demonstrated in a mice model. It is intriguing, that all three pathogens may also utilize common receptors amid the hepatocyte invasion [3]. Furthermore, the impact of HBV and HCV infection on the clinical picture of malaria has not been adequately addressed. In this review, we aimed at putting together published data and analyzing it in order to come out with a clear picture about the pathophysiology, clinical presentation, and future prospects [2]. Due to the coincidence in the endemicity of HBV and Plasmodium infections, co-infection of HBV with Plasmodium may occur [4]. These two infections live some of their developmental stages within the liver, and hepatocyte damage in HBV infection may cause poor liver handling of malaria parasites [5] thus culminating in increased morbidity. In line with this, co-infection with Plasmodium parasite and HBV virus in individual may possibly influence progression of both agents and associated severity. It is on this background that this work determines the sero-prevalence of Hepatitis B Virus profile and Plasmodium co-infection among patients attending hospitals in Wukari environs [3]. Due to low vaccination rates and the fact that about 75% of the Nigerian population will be exposed to HBV [6], the risk of contracting HBV is quite significant. In a previous study conducted in a University located in Wukari, a Hepatitis B Surface Antigen (HBsAg) seroprevalence rate of 6.0% was observed [1]; which raises some level of concern. On the other hand, Nigeria is known for having the world's greatest malaria burden, recording approximately 51 million cases and 207,000 deaths annually, while 97 % of the total population is at risk of infection [7]. Worthy of mention is the fact that malaria accounts for 60% of outpatient visits to hospitals [7]. Malaria and HBV infection are co-endemic throughout much of the tropical and Sub-Saharan Africa and they both present major threat to public health [8]. These facts are quite worrisome and even more alarming when HBV infection occurs concurrently with malaria. In 2018, the World Health Assembly resolved to recognize viral hepatitis as a global health problem [9]. There remains a scarcity of research on people's knowledge, attitudes, and practices towards malaria in the majority of the federation, particularly in Northern Nigeria [10]. Also, not much study has been carried out on HBV in most parts of the country and much is not known about its epidemiology [1]. This study therefore became necessary, in that, it will describe the epidemiology of HBV and HCV and highlight the burden of the HBV, HCV and malaria co-infection in Wukari, identify gaps and make recommendations for improvements on surveillance and control of both infections. This study is therefore necessary to determine the Immunological evaluation of Hepatitis B Virus, Hepatitis C and Plasmodium co-infection profile among patients in Wukari in the North East region of Nigeria.

2. Materials and Methods

2.1. Study area and population

This study was carried out in the Department of Microbiology, Federal University Wukari, Taraba State, Nigeria. Wukari metropolis is a large town which is the Headquarter of Wukari Local Government Area of Taraba State. Geographically, Wukari lies between latitude 7°55'42" North and longitude 9°47'59" East. It has an area of 4,308 km2. Wukari is home to Federal University Wukari, National Open University of Nigeria study center and Kwararafa University. It is an important town in Taraba state, characterized with agricultural activities among other activities. The average annual temperature in Wukari is 26.8°C, with March being the hottest month with an average temperature of 29.8°C and August has the lowest average temperature with 25.4°C. The average precipitation is 1205mm. The major languages spoken are Jukun, Kutep, Tiv, Hausa and Fulani [1].

2.2. Ethical and Consent approval

A letter of introduction was obtained from the Department of Microbiology, Federal University Wukari, and ethical approval was obtained from the hospital management and Taraba State Ministry of Health. The purpose and procedure of the study was explained to the volunteers, parents or caregivers and their consent was obtained for samples collection. Written consent has been collected and preserved by the author(s).

2.3. Study design

The study design is a community, hospital-based cross-sectional and experimental study. The essence of the research was explained to the target population and their consents were obtained after which blood samples were collected.

2.4. Sample collection

All materials (sterile tubes, syringes and needles, tourniquet, cotton swab) required for the collection of venous blood were assembled and labeling of the sterile plain containers with the subjects' identification numbers were done. About 5ml of blood samples were collected from one hundred (100) participants aseptically by venipuncture and dispensed into sterile labeled tubes with anticoagulants. Thick smears of the blood samples were made at the spot on clean and grease free slides. The blood samples were transported in an ice box to the laboratory. The whole blood was stored at 4-8°C in the refrigerator for up to 24 hours, but was not allowed to freeze.

2.5. Analysis of samples for Plasmodium SPP

Analysis was performed using microscopic examination of thick smears and parasitemia (parasites/mL of blood) was calculated in positive cases. Malaria Rapid Diagnostic Test (RDT) was performed using whole plasma samples from all individuals to confirm diagnoses, using a test kit from Standard Diagnostics, Inc.

2.5.1. Procedure for Thick film

The procedure for staining and viewing thick film to establish malaria parasitaemia was done according to the method described by [11].

2.5.2. Rapid Diagnostic Test Procedure
The procedure was carried out based on the manufacturer's instruction. All the kit components and specimens were brought to room temperature prior to testing. The test device was removed from the foil pouch and placed on a flat, dry surface. It was labeled with subject's ID number. About 5µL of the blood sample was dispensed into the round specimen well. Four drops of assay diluent were placed into the assay diluent well. After a range of 15 to 30 minutes, the results were read.

2.5.3. Interpretation of Result
Negative: One line "C" in the result window indicates that no Malaria P. falciparum antigen is present. Positive: Two lines "C" and "P.f" in the result window indicates P. falciparum positive. Invalid: No "C" line in the result window

2.6. Analysis of Samples for Hepatitis B Virus and Hepatitis C Virus
Serum samples were analyzed for the presence of HBV Surface Antigen (HBsAg), Surface Antibody (HBsAb), Core Antibody (HBcAb), Envelope Antigen (HBeAg) and Envelope Antibody (HBeAb) using HBV Combo Rapid Test Cassette by Acro Biotech, Inc. The analysis for HVC was done using TELL Rapid diagnostic strip, a commercially available test kits for the detection of anti – HCV antibody from each patient's serum. The HCV Rapid Test strip is a qualitative, membrane based immunoassay for the detection of antibody to HCV in serum or plasma.

2.6.1. Procedure for Hepatitis B profile test
The test was carried out using the procedure outlined by the manufacturer of the kit used. All the kit components and specimens were brought to room temperature prior to testing. Clear plasma was separated from the blood which showed no sign of haemolysis. The test cassette was removed from the foil pouch and placed on a clean and level surface. It was labeled with subject's ID number. Holding the dropper vertically, 3 drops of the plasma sample were dispensed into each sample well of the test cassette respectively, without trapping air bubbles. The results were read within 15 to 20 minutes, but not later than 20 minutes.

2.6.2 Interpretation of Results
2.6.3. For HBsAg, HBsAb, HBeAg
Negative: One coloured line appears in the control line (C) and no apparent coloured line appears in the test region (T). Positive: Two distinct coloured lines appear, one at the control region (C), the other at the test region (T). Invalid: Control line fails to appear. For HBeAb, HBcAb, Negative: Two distinct coloured lines appear, one at the control region (C), the other at the test region (T). Positive: One coloured line appears in the control region (C) and no apparent coloured line appears in the test region (T). Invalid: Control line fails to appear.

2.6.4 Interpretation of Results for Hepatitis C virus
Negative: A red/pink line appears in the control line (C) and no apparent colored line appears in the test region (T). Positive: Two red/pink lines appear, one at the control region (C), the other at the test region (T). Invalid: Control line fails to appear or no line appears at all.

2.7 Data/Statistical Analysis
The sero-prevalence of HBV and HCV infection was calculated by using participants with positive sample as numerator and the total number of participant as denominator. The data obtained from this study were presented using descriptive statistics.

3. Result
Out of the total 100 blood samples screened for Hepatitis B virus, Hepatitis C virus and malaria infections among patients, 15 patients were found to be positive revealing a prevalence rate of 15%, for Hepatitis B virus, 6 patients were found to be positive revealing a prevalence rate of 6%, for Hepatitis C virus and 2 patients were found to be positive revealing a prevalence rate of 2%. The studies subjects shows that 34 blood samples were males and 66 of them were female. Of this, 6 (17.0%) males were sero-positive for HBsAg, while about 94 (94% of 100) were sero-negative of the study population (100). This is shown in table 1. Table 2 shows the distribution of HBV and HCV carriers according to sex and age groups. The highest prevalence of HBV was within age group of 25-34, 4 (4.0%) for male and 25-34, 4 (4.0%) for female, while, HCV has the least prevalence age group of 15-24, 1 (10%), 25-34, 1 (10%), for male and age group of 15-24, 1 (10%), 25-34, 1 (10%) and 35-44, 1 (10%) for females. The male population was the only one tested positive for malaria parasite from the age group of 15-24, 2 (2.0%). Table 3 shows the distribution of patients based on gender and age group. Figure 5 shows the sero-positive of HBV with the highest value among ages 25-34 in males. Figure 6 shows the distribution of HCV sero-prevalence by age group and sex.

Table 1: Sero-Prevalence of HBV and HCV by sex among patients

Sex	HBsAg	HCV	HBsAg	HCV	HBsAg	HCV
Male	34	34	6(17.0%)	2(5.0%	28(82.0%)	32(94.0%)
Female	66	66	9(13.0%)	4(6.0%)	57(86.0%)	62(93.0%)
Total	100	100	15(15.0%)	6(6.0%)	85(85.0%)	94(94.0%)

Table 2: Distribution of HBV and HCV carriers according to sex and age group

	Sex		Male No. positive (%)		Female No. Negative (%)	
Age	**Male**	**Female**	**HBV**	**HCV**	**HBV**	**HCV**
15-24	18	7	2(11.0%)	1(55.0%)	3(42.0%)	1(14.0%)
25-34	8	44	4(5.0%)	1(12.0%)	4(90.0%)	0(0%)
35-44	2	9	0(0%)	0(0%)	1(11.0%)	1(11.0%)
45-54	3	1	0(0%)	0(0%)	1(10.0%)	0(0%)
55-64	2	3	0(0%)	0(0%)	0(0%)	0(0%)
65-74	1	2	0(0%)	0(0%)	0(0%)	1(5.0%)

Table 3: Distribution of patients based on malaria positivity and Negativity according to sex Gender

Subject	Males n.(%)	Females n.(%)	Total
Malaria positive	2(58%)	0(0%)	2
Malaria negative	32(94%)	66(100%)	98
Total	34 (100%)	66(100%)	100

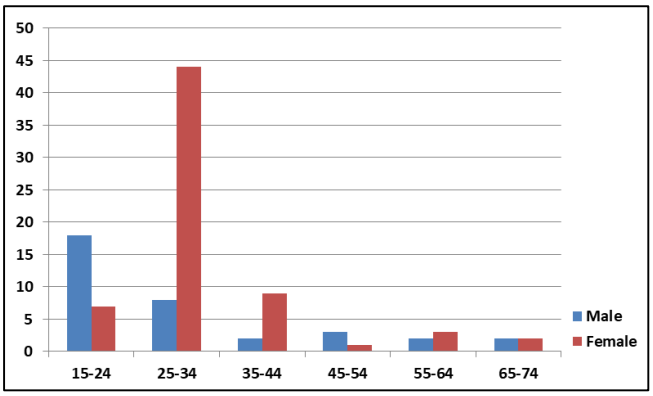

Fig 1: Bar chart showing the distribution of HBV carrier by sex and age group

4. Discussion

In this research, an overall sero-prevalence rate of 15% for HBsAg was recorded during the study period; a sero-prevalence rate of 6.0% was recorded for anti-HCV with no case of co-infection. The 15% for HBV in this study is higher than that of [12] which had a prevalence rate of 4.7% among students in University of Uyo. [13] recorded a prevalence of 11.0% among medical students of Makerrere University Uganda. In ABU Zaria, prevalence rate of 12. 5% was recorded among students [14], prevalence rate of 12.8% in Maiduguri, 11% in Makurdi [15]. In the University of Ilorin, 8.0% prevalence rate was reported by [16]. This prevalence rate of 15% for HBV in this research falls below the category of high endemicity which states 'high endemicity for HBV infection as HBsAg values is greater than 7% in an adult population' [2]. The prevalence rate of 6.0% for HCV in this research is comparatively higher than the 1.3% seroprevalence reported by [17], with the same Cypress anti-HCV dipstick [17] and also higher than the 5.3% reported for the whole African region by WHO and a lower prevalence of 2.0% among the general population of Nigeria [9]. The prevalence of HBV and HCV differs in or varies in different population groups [18]. The prevalence of 0.6 to 2% was reported in western countries and ≤15% in other regions of the world [18]. African blood donors have been reported with estimated prevalence of 6.0% compared to 0.5 – 15% seen in blood donors in Europe and North America [19]. This present research recorded a significant incidence of HBV and HCV infections amongst patients in Wukari Local government area, Nigeria. The result of this study showed 2% malaria prevalence among patients, which is lower than the prevalence of the general population (2.8%) in Nazareth, South West Ethiopia [2]. Although much is known about the epidemiology of HBV and HCV in Nigeria, limited investigations have been carried out on HBV and HCV in some parts of the country. Studies carried out by various authors have shown that HBV and HCV infections are highly prevalent among Nigerians [1].

5. Conclusion

Conclusively, the result of this study which shows that the prevalence of HBV (15%) and HCV (6%) which is high when compared with the study population shows that many of these individuals do not know much about hepatitis viral infection, mode of transmission, vaccination, treatment, prevention and control, hence the high prevalence rate recorded in this study population. As a result of this, there is need for adequate screening programme to reduce the transmission of the virus. Public awareness programme to educate the patients on modes of transmission should be put in place for HBV vaccination because many of the patients are not aware of the infection and its mode of transmission, some are afraid of taking vaccine for various reasons such as religion which in this part most people still believe that the use of drugs is against their religion. Therefore, in order to reduce HBV and HCV infection, mass immunization of adults and antiviral drugs should be provided for those that are infected, while HBV and HCV screening programs should be instituted in all communities in the country to reduce the prevalence rate and level of transmission of the hepatitis virus. Screening of blood donors for both HBV and HCV markers independently is recommended. Moreover, a 2% prevalence of malaria parasites in blood donors requires greater concern particularly in malaria endemic areas and it is also recommended that physicians or blood collectors need to ask blood donors for history of recent malaria infection and need to confirm negativity by laboratory tests according to the reply of the blood donor before donating blood. The failure of serological screening tests to identify recently infected blood donors requires the use of advanced tests to reduce the risk of transmitting hepatitis viruses through blood transfusion during the window period. A prevalence of 15% HBsAg and 6.0% anti-HCV might warrant the introduction of screening of all blood donors for hepatitis viral markers (HBV and HCV) and should be instituted in Wukari Local Government Area as well as in the Local Government Area, if not all communities in general. Formulation of safe blood transfusion policy and implementation of standard laboratory screening procedures at all levels is strongly recommended.

Competing Interests

Authors have declared that no competing interests exist.

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