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Hepatitis B and C Surface Antigens and Typhoid Fever in Voluntary Non – Remunerated Blood Donors in Abakaliki, Nigeria

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Abstract: Infections due to Hepatitis B and Hepatitis C Viruses (HBV and HCV) are worldwide significant problems in public health. This cross-sectional and comparative study was carried out to evaluate and compare the prevalence of HBV, HCV and typhoid bacteria among voluntary non-remunerated blood donors in Abakaliki, Nigeria. A total of 307 voluntary non-remunerated blood donors were screened using rapid test (Global Rapid Diagnostic Kit, USA) for the detection of HBsAg and anti-HCV in serum samples. The positive samples were screened using ELISA Kit (Clinotech Diagnostics, Canada) to check the specificity of the screening test for HBV and HCV. The results obtained showed that 20(6.52%) and 10(3.26%) were seropositive to HBsAg and anti-HCV respectively. The prevalence of HBsAg and HCV were higher in males 17(5.54%) and 6(1.95%) compared to 3(0.98%) and 4(1.30%) in females respectively. Age specific prevalence of HBV was higher in the age brackets 15-24 and 25-34 years with 4(6.67%), and 14(8.33%), lower in the age bracket 35-44 with 2(2.70%) and lowest in the age bracket 45+ years with 0(0.00%). Age specific prevalence of HCV was higher in the age brackets 25-34 and 35-44 years with 7(4.17%) and 3(4.05%) respectively and lowest in the age brackets 15-24 and 45+ years with no cases at all among them. Statistically, there was no significant relationship between age, HBV and HCV infections ($p > 0.05$). The most important risk factors in the acquisition of HBsAg as revealed in this study appears to be: those with multiple sexual partners, 12(20.00%), family history of hepatitis, 4(15.39%), blood transfusion 5(9.62%), and tribal mark/tattooing, 2(6.25%) while the most important risk factors in the acquisition of HCV appears to be: family history of hepatitis, 5(19.23%), and blood transfusion 4(7.69%). In comparison, HBV is more prevalent among blood donors in Abakaliki than HCV. Furthermore, this study also reveals that few blood donors have bacteraemia, thus the prevalence of typhoid bacteria among blood donors is very low. The intermediate prevalence of HBV and HCV, and low prevalence of typhoid bacteria recorded among blood donors in this study are probably a reflection of situation in Abakaliki, Nigeria. Therefore, urgent-preventive measures should be taken to set up campaign against transmission of HBV and HCV in Abakaliki, Nigeria. To lower hepatitis prevalence, program of active screening and vaccination for voluntary non-remunerated blood donors is recommended.

Keywords: HBV, HCV, Typhoid Bacteria, Voluntary Non-Remunerated, Blood Donor

1. Introduction

Hepatitis B virus (HBV) and Hepatitis C Virus (HCV) are among the most transfusion transmissible infectious agents. Hepatitis is an inflammation of the liver characterized by the presence of inflammatory cells in the tissue of the organ. It may occur with limited or no symptoms, but often leads to jaundice, anorexia (poor appetite) and malaise. Hepatitis is acute when it lasts less than six months and chronic when it persists longer [1]. A group of viruses known as the hepatitis viruses cause most cases of hepatitis worldwide, but it can also be due to toxins (notably alcohol, certain medications

and plants), other infections and autoimmune diseases [2]. The hepatitis virus is found in the blood and other body fluids and is transmitted from person to person. The most common routes of infections include blood transfusions and blood products, medical or dental interventions in countries where equipment is not adequately sterilized, mother to infant during child birth, sexual transmission (in the case of hepatitis B), sharing equipment for injecting drugs, sharing straws, notes etc. for snorting cocaine, sharing razors, tooth brushes, or other household articles, tattooing and body piercing if done using unsterile equipment. Hepatitis B is spread through contact with the blood or other body fluids (i.e. semen, vaginal fluid and saliva) of an infected person while hepatitis C virus is spread through direct contact with infected blood. Very rarely hepatitis C virus can also be passed on through other body fluids. Many people infected with hepatitis B or C rarely displays any symptom, although they can still transmit the virus to others [3]. Hepatitis B is a major disease of serious global public health proportion. It is preventable with safe and effective vaccines that have been available since 1982. Of the 2 billion people who have been infected with the hepatitis B virus (HBV) globally, more than 350 million have chronic (lifelong) infections [4]. Over 20 million people are infected annually with this virus [5]. Hepatitis C is the most common blood-borne (direct contact with human blood) infection. The world health organization (WHO) estimates that about 3% of world populations (200 million peoples) have hitherto been infected with the Hepatitis C virus [6]. Blood transfusion is increasingly becoming a major mode of transmission of HBV and HCV especially in high prevalence areas such as sub-Saharan Africa. There is a high level of occurrence of blood demanding health condition in many parts of sub-Saharan Africa. The increase in road accidents, pregnancy- related haemorrhage, anaemia due to disease conditions and malnutrition, armed conflicts, and violent events in the sub-region increase the possibility of the emergency transmission of HBV and HCV through contaminated blood.

This study is therefore aimed at investigating and comparing the serum prevalence of hepatitis B and C viruses among voluntary non – remunerated blood donors in Abakaliki, Nigeria. It will also analyse donors' blood for typhoid bacteria (*Salmonella typhi*) and screen isolates for drug resistance using Federal Teaching Hospital Abakaliki (FETHA) and National Obstetrics Fistula Centre (NOFIC), Ebonyi State as study sites. The study is a hospital based cross sectional and comparative study that will include voluntary non-remunerated blood donors in NOFIC and FETHA, Ebonyi State, Nigeria.

2. Materials and Methods

2.1. Research Design

The study is a cross- sectional and comparative study design to obtain information on the prevalence of Hepatitis B virus, Hepatitis C virus and typhoid bacteria (*Salmonella typhi*) among voluntary non- remunerated blood donors in Abakaliki, Ebonyi State, Nigeria.

2.2. Study Area

The study was conducted at the Federal Teaching Hospital Abakaliki and National Obstetric Fistula Centre, located at the centre of Abakaliki, Nigeria. Abakaliki is the capital city of the present day Ebonyi State in South-eastern Nigeria, located 64 kilometres (40 mi) south-east of Enugu. It is situated at 6.32° North latitude, 8.12° East longitude and 117 meters' elevation above the sea level. It is a big town in Nigeria, having about 134,102 inhabitants (Map data ©2016 google).

Figure 1. *Map of Abakaliki, Ebonyi State showing Federal Teaching Hospital Abakaliki (FETHA).*

2.3. Ethical Consideration

Ethical approval was sought and obtained from the Research and Ethical Committee of the Federal Teaching Hospital Abakaliki (FETHA) and National Obstetric Fistula Centre (NOFIC) Abakaliki, Ebonyi State respectively. Consenting voluntary non-remunerated blood donors were assured of strict confidentiality of any information given.

2.4. Study Population

Three hundred and seven (307) consented voluntary non-remunerated blood donors were used for this study. Of the three hundred and seven participants, two hundred and forty-six (246) were males and sixty-one (61) were females. Age, history of blood transfusion, and health record were documented for each individual donor.

2.5. Sample Selection and Laboratory Study

The study was done between July, 2015 and August, 2017. Five millilitres of blood was collected from each donor into a plain tube by vein puncture using a sterile syringe and needle. Each sample was properly labelled with the number corresponding to number assigned to consenting participant. The participating donors were interviewed by issuing them questionnaire, to obtain information on their social-demographic data such as age, sex, history of blood or blood products transfusion, etc. Samples collected were allowed to clot and retract, after which serum was obtained by centrifuging at 3000 revolutions per minute (rpm). The serum was separated from whole blood and stored at -20ºC for further analysis. In the laboratory, samples were tested for the presence of HBV, HCV and typhoid infections using the adsorption qualitative techniques based on the principles of antibody reaction (Global diagnostic kit USA) for virus and culture technique for typhoid bacteria at the Federal Teaching Hospital and National Obstetric Fistula Centre Laboratories Abakaliki, Ebonyi State.

2.6. Global Diagnostic one Step HBsAg and Anti – HCV Test Kits Assay Procedure

The one step hepatitis B surface antigen and anti-HCV test strips (serum/plasma) are rapid chromatographic immunoassay for the qualitative detection of hepatitis B surface antigen and hepatitis C virus antibody respectively, markers of Hepatitis B and C infections in serum/plasma specimens or whole blood specimens. The test strips and serum were brought to room temperature before the tests were carried out. The test strips were removed from the pouch and labelled appropriately with arrows pointing toward the serum specimen. The test strips were immersed vertically in the serum for 10-15 seconds making sure that the maximum line (MAX) in the test strips were not exceeded when immersing the strips. The test strips were then removed after the appropriate time and placed on a non-absorbent flat surface. The results were read after 15 minutes.

2.7. Enzyme Linked Immuno Assay (ELISA) Technique

This test was carried out on the positive specimens using ELISA kits (Clinotech Diagnostic, Canada), to check the specificity of the screening test for HBV and HCV. In ELISA techniques to detect antibody, antigen is bound to the cell well of micro titration plate or filter membrane (EIA membrane test) and the patient's serum added. Antibody binds to the antigen. After washing, anti- human immunoglobulin (AHG) conjugated with an enzyme is added which binds to the antibody- antigen complex. After a further wash, a chromogenic enzyme substrate is added, producing a colour reaction which is read spectrophotometrically (micro titration plate EIA) or visually (membrane EIA) [7].

2.8. Culture, Isolation, and Identification of Salmonella Typhi

About 5ml of blood sample were added to blood culture bottles containing 50ml of Brain Heart Infusion (BHI) broth, the Brain Heart Infusion broth contain 0.05% sodium polyanethol sulphonate (SPS). The blood culture bottles were incubated at 37ºC for 24h. The broth was sub-cultured on blood media after 1^{st}, 2^{nd}, 3^{rd} - - - and 7^{th} day. Isolates were Gram stained and identified by standard biochemical methods [7]. The broth that had no growth after 10days, were considered negative culture. The isolates were identified on bases of their cultural and morphological characteristics on growth media. Discrete colonies from sub-cultured isolates were Gram-stained to differentiate the isolates based on their Gram staining reaction. Biochemical tests such as; indole production, citrate utilization, urease production, H_2S production and Lysine decarboxylase, Triple sugar ion agar (TSIA) test were carried out as a confirmatory test [7]. The isolates were sub-cultured and maintained on a slope of nutrient agar in a universal bottle and kept at 4ºC in the refrigerator for further analysis.

2.9. Antibiotic Susceptibility Testing

Antibiotic sensitivity tests were carried out on all isolates considered to be *S. typhi*. Susceptibility testing was done by the disc diffusion method in accordance with CLSI [8]. Isolates were tested against a panel of eight antibiotics (Abtek Liverpool, UK): Augmentin (30µg), Nitrofurantoin (20µg), Nalidixic acid (30µg), Cotrimoxazole (25µg), Gentamycin (10µg), Amoxicillin (25µg), Ofloxacin (5µg) and Tetracycline (25µg). Inhibition zone diameters were measured in millimeters and susceptibility scored as resistant, intermediate or sensitive, according to CLSI guidelines.

2.10. Statistical Analysis

All analyses were performed using SPSS 20; significant difference was determined using chi-square at 95% CI. The results of the analyses were presented in simple percentages and tables for easy comprehension.

3. Results

Of the 307 consented voluntary non-remunerated blood donors screened, 20(6.52%) were seropositive for HBsAg, while 10(3.26%) were seropositive for anti- HCV. Of the twenty (20) and ten (10) samples that tested positive for HBV and HCV, 17(5.54%) and 6(1.95%) were males while 3(0.98%) and 4(1.30%) were females. The prevalence of HBsAg was higher in males, 17(5.54%) compared to 3(0.98%) in females. While the prevalence of HCV was also higher in males 6(1.95%) compared to 4(1.30%) in females. Age specific prevalence of HBsAg was higher in the age brackets 15-24 and 25-34 years with 6.67%, (n = 4) and 8.33%, (n = 14), low in the age brackets 35-44 years with 2.70%, (n = 2), while the age specific prevalence of HCV was higher in the age brackets 25-34 and 35-44 years with 4.17%, (n = 7) and 4.05%, (n = 3) respectively, and age brackets 15-24 had no cases, also age brackets 45+ had no cases at all among them in both. There was no statistically significant relationship between the HBV and HCV infections, age and gender ($p > 0.05$), as shown in (Table 1).

Table 1. *Prevalence of HBV and HCV infections among blood donors by Age and Gender.*

Age groups (years)	Total No of participant	Male			Female			Total No (%) positive of HBV	Total No (%) positive of HCV
		No of participants	No (%) positive of HBV	No (%) positive of HCV	No of participants	No (% of positive of HBV	No (%) positive of HCV		
15 – 24	60	37	4(10.81)	0(0.08)	23	0(0.00)	0(0.00)	4(6.67)	0(0.00)
25 – 34	168	137	11(8.03)	4(2.92)	31	3(9.68)	3(9.68)	14(8.33)	7(4.17)
35 – 44	74	67	2(2.99)	2(2.99)	7	0(0.00)	1(14.29)	2(2.70)	3(4.05)
45^{+}	5	5	0(0.00)	0(0.00)	0	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Total	307	246	17(5.54)	6(1.95)	61	3(0.98)	4(1.30)	20(6.52)	10(3.26)

Age (HBV): $X^2 = 6.021$, P-value = 0.537; Gender: $X^2 = 0.319$, P-value = 0.572
Age (HCV): $X^2 = 9.695$, P-value = 0.207; Gender: $X^2 = 2.631$, P-value = 0.105

The following risk factors: Multiple sex partners, Family history of hepatitis, Blood transfusion and Tattoo/ Tribal mark associated with HBV transmission were examined. The results showed the prevalence rates of 20.00%, 15.39%, 9.62% and 6.25% respectively. There were statistically significant association between the HBV transmission and risk factors (family history of hepatitis, multiple sexual partners and blood transfusion) ($p < 0.05$), but there was no statistically significant association between the risk factor (Tattoo/ Tribal mark) and acquisition of HBV infection ($p > 0.05$), as shown in Table 2.

Table 2. *Prevalence of HBV among blood donors by Risk factors.*

Risk factor	No. of Participants	No. positive	Prevalence (%)	$\chi 2$ P- values
Family History of Hepatitis				
Yes	26	4	15.39	2.0631
No	281	16	5.69	0.0105
Sub total	307	20	6.52	
Multiple Sex Partners				
Yes	60	12	20.00	2.0631
No	247	8	3.24	0.0105
Subtotal	307	20	6.52	
Blood Trans-fusion				
Yes	52	5	9.62	0.988
No	255	15	5.88	0.0320
Sub total	307	20	6.52	
Tattoo/Tribal Mark				
Yes	32	2	6.25	0.004
No	275	18	6.55	0.949
Sub total	307	20	6.52	

The most important risk factors in the acquisition of HCV as revealed in this study appears to be, family history of hepatitis, multiple sex partners, blood transfusion and tattoo/ tribal mark. The results showed the prevalence rates of 19.23%, 0.00%, 7.69% and 0.00% respectively. There was a statistically significant association between the HCV transmission, family history of hepatitis and blood transmission ($p < 0.05$), but there was no statistically significant association between the HCV acquisition, multiple sex partners and tattoo/ tribal mark ($p > 0.05$), (Table 3).

Table 3. *Prevalence of HCV among Blood Donors by Risk Factors.*

Risk Factor	No. of Participants	No. positive	Prevalence (%)	χ^2 P- values
Family History of Hepatitis				
Yes	26	5	19.23	23.000
No	281	5	1.78	0.000002
Sub total	307	10	3.26	

Risk Factor	No. of Participants	No. positive	Prevalence (%)	χ^2 P- values
Multiple Sex Partners				
Yes	60	0	0.00	2.511
No	247	10	4.05	0.113
Subtotal	307	10	3.26	
Blood Transfusion				
Yes	52	4	7.69	3.908
No	255	6	2.35	0.048
Sub total	307	10	3.26	
Tattoo/Tribal Mark				
Yes	32	0	0.00	1.203
No	275	10	3.64	0.273
Sub total	307	10	3.26	

The highest prevalence of HBV was found among those within the age brackets of 25-34 and 15-24 years with 8.33%, (n = 14) and 6.67%, (n = 4) respectively and the highest was observed within the age bracket 25-34 years with 8.33% prevalence rate while the age specific prevalence of HCV infection was higher within the age brackets of 25-34 and 35-44 years with 4.17%, (n = 7) and 4.05%, (n = 3) respectively and the highest was observed within the age bracket 25-34 years with 4.17%, as shown in Table 4 below.

Table 4. *Comparison of HBV and HCV prevalence among blood donors by Age.*

Age groups (years)	No. of Participants	No. (%) Positive of HBV	No. (%) Positive of HCV
15 – 24	60	4 (6.67)	0 (0.00)
25 – 34	168	14 (8.33)	7 (4.17)
35 – 44	74	2 (2.70)	3 (4.05)
45+	5	0 (0.00)	0 (0.00)
Total	307	20 (6.52)	10 (3.26)

This study revealed that males had the highest rate of HBV and HCV infections with 17 (5.54%) and 6 (1.95%) respectively compared to the females with 3 (0.98%) and 4 (1.30%), as shown in Table 5 below.

Table 5. *Comparison of HBV and HCV Prevalence among Blood Donors by Gender.*

Gender	No. of Participants	No. (%) Positive of HBV	No. (%) positive of HCV
Male	246	17 (5.54)	6 (1.95)
Female	61	3 (0.98)	4 (1.30)
Total	307	20 (6.52)	10 (3.26)

Table 6. *Comparison of HBV and HCV Prevalence among Blood Donors by Risk Factors.*

Risk Factor	HBV Prevalence (%)	HCV Prevalence (%)
Family History of Hepatitis		
Yes	15.39	19.23
No	5.69	1.78
Multiple sex partners		
Yes	20.00	0.00
No	3.24	4.05
Blood Transfusion		
Yes	9.62	7.69
No	5.88	2.35
Tattoo/ Tribal mark		
Yes	6.25	0.00
No	6.55	3.64

Based on our findings, the most important risk factors in the acquisition of HBV infection appear to be; Family history of hepatitis, multiple sex partners and Blood transfusion while the most important risk factors in acquisition of HCV infection appear to be; Family history of hepatitis and blood transfusion, as shown in Table 6.

The specificity of the screening test for HBV and HCV using ELISA kits was checked, the results revealed that rapid immmunochromatographic strips used for HBV and HCV testing were specific, since the twenty (20) and ten (10) blood samples that tested positive for HBV and HCV respectively when immmunochromatographic strips were used, also tested positive with Enzyme Linked Immune Absorbent Assay Kits (ELISA Kits).

Out of the three hundred and seven (307) donors blood sample cultured, only two (2) blood samples; BS28, BS118 showed positive growth for typhoid bacteria and was isolated and identified as *Salmonella typhi* (two isolates). The isolates were susceptible to Gentamycin and Amoxicillin and were resistant to Ofloxacin, Augmentin, Nalidixic Acid, Nitrofurantoin, Cotrimoxazole and Tetracycline.

4. Discussion

Infections due to Hepatitis B and Hepatitis C Viruses (HBV and HCV) are worldwide significant problems in public health [9-10]. About 5% (300 million), of world population has chronic HBV infection, which is the major factor for the development of chronic liver cirrhosis and hepatocellular carcinoma [11-12]. The prevalence of HCV in world level can be more than 3% [13-15]. Out of 307 samples of the voluntary non-remunerated blood donors tested, 20 (6.52%) and 10 (3.26%) were seropositive for HBsAg and Anti-HCV. The HBV and HCV prevalence rate among voluntary non- remunerated blood donors in Abakaliki according to this study is 6.52% and 3.26%. In comparison to studies from other parts of the country, the prevalence rate reported in this study is less than the 23.9 and 21.3% recorded in studies in Jos and Ibadan [16-19]. The difference may be due to sample size, and increased awareness about HBV and HCV. The difference can also be due to the fact that subjects involved in this study are not high risk groups for infection with HBV and HCV. Ugwuja and Ugwu, (2008) in their report on the seroprevalence of Hepatitis B surface antigen and liver function tests among adolescents in Abakaliki, South East, Nigeria, reported a prevalence rate of 3.9% (low intermediate prevalence), which is similar to our findings of a prevalence rate of 3.26% for HCV.

Based on our findings, HBV infection is more prevalent among voluntary non- remunerated blood donors with 20 (6.52%) infections than HCV infection with 10 (3.26%) infections. The reason for higher prevalence of HBV among voluntary non- remunerated blood donors compared to HCV prevalence in Abakaliki according to this study could be because of their modes of transmission; HBV is spread via mother to child at birth (perinatal), contact with an infected person (horizontal), sexual contact and exposure to blood or other infected fluids [20-21] while HCV is spread through direct contact with infected blood. HBV infection by gender distribution in this present study showed that males had higher HBV infection rate (5.54%) than their female counterparts (0.98%). In HCV infection, a higher prevalence rate was found in males (1.95%) when compared to their female counterparts (1.30%). Statistically, there was no significant association between gender and HBV and HCV infections, ($p \rangle 0.05$). This findings differs from the report of Olakayode *et al*. (2009), who reported 17.5% and 10.5% for males and females, in their report on HBV infection among intending blood donors in Ibadan, Nigeria. The reason for high infection rate among males may be due to habits such as multiple sex partners and polygamy which may be higher among the males. In Nigeria, multiple sexual partnership and proximity are habits occurring with higher frequency among males than females as indicated by UNSN [22].

Our study found a higher prevalence rate of HBV infection among people within the age group of 15-24 (6.67%) and 25-34 (8.33%). The reason for the higher prevalence rate of HBV among the age groups may be due to high sexual transmission among members of the age group [23]. In HCV infection, a higher prevalence rate was found among those within the age groups 25-34 and 35-44 years with 4.17% and 4.05%. This is in accordance with the review by CDC, (1998) showing that about 3.9 million people worldwide are infected with HCV, with the highest among age group 30-39 years.

The factors associated with increased risk of HBV and HCV include duration of sexual activity, and number of sexual partners. Clients or prostitutes are at particularly high risk for HBV infection among the age groups. Considering the risk factors associated with HBV and HCV infections, our finding shows that the major risk factors in transmission is, having a history of hepatitis infection in the family (15.39% and 19.23%), multiple sex partners (20.00%), blood transfusion (5.62% and 7.69%), and tattooing/tribal marking (6.25%). The intermediate prevalence rate of HBV (6.52%) and HCV (3.26%) among voluntary non- remunerated blood donors in this study shows that hepatitis B virus and hepatitis C virus are endemic among voluntary non-remunerated blood donors in Abakaliki, Ebonyi State. This study also reveals that few of the voluntary non- remunerated blood donors that participated in the study had typhoid bacteria isolated from their blood samples and were identified as *Salmonella typhi*. The prevalence of typhoid bacteria among voluntary non-remunerated blood donors in Abakaliki, according to this study was very low. The reason for the low prevalence of typhoid bacteria could be because of its transmission route; typhoid fever (*Salmonella typhi*) is transmitted via oral route by ingestion of food or water contaminated with faeces [24], and is mostly detected by faecal culture.

5. Conclusion

This study has provided information on the burden of Hepatitis B and C surface antigens and typhoid fever in voluntary non-remunerated blood donors in Abakaliki. The total prevalence of HBV and HCV among voluntary non remunerated blood donors in Abakaliki, according to this study are 6.52% and 3.26% respectively. Higher prevalence rate was observed in HBV than HCV, and lowest was observed in typhoid bacteria. The implication of high prevalence of asymptomatic HBV and HCV infections among blood donors are that they may become chronic carriers of the virus, thus acting as reservoirs for subsequent transmission to others (recipients).

Infection caused by *Salmonella typhi* remains an important public health problem, particularly in developing countries. Morbidity and mortality attributable to typhoid fever are once again increasing with the emergence and worldwide spread of *Salmonella typhi* strains that are resistant to most previously useful antibiotics. Base on these findings, there is renewed interest in understanding the epidemiology of hepatitis B virus, hepatitis C virus and typhoid fever in voluntary non remunerated blood donors in Abakaliki, and Nigeria at large.

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