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Assessment of Impact of HBV Markers on Haematological and Hepatic Parameters on Seropositive Blood Donors in 2014 at ABUTH Zaria, Kaduna State.

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ABSTRACT

AIMS: To determine the pattern of HBV serologic markers among HBsAg sero-positive prospective blood donors (PBD) and to assess the derangement in biochemical liver enzymes and some haemtological parameters with a view towards developing a blood donor policy.

MATERIALS and METHODS: A clinic-based study of PBDs between the ages of 18 to 65 years. 100 PBD tested positive to HBsAg considered as "subjects" were tested for HBsAb, HBeAg, HBeAb and HBcAb by rapid antibody determination.100 HBsAg Sero-negative donors were used as "Controls". Complete blood counts and liver enzymes were carried out on all participants by automated and manual techniques respectively. Data analysis was conducted with Graph Pad Prism 6 statistical software, and findings presented as frequency distribution, means, standard deviation, bar charts and Student T Test to compare the Subject's and Control's where appropriate, p value of ≤ 0.05 was considered as significant.

RESULTS: Analysis of serological HBV surface markers among the subjects showed a positivity rate of 11% for HBsAb, 14% HBeAg, 74% HBeAb, and 99% of HBcAb, suggesting a high rate of acute, chronic and occult infection but a low rate of infectivity. All the liver enzymes and Prothrombin time were statistically significant,p value < 0.0001 elevated in the subjects. However, the blood cell counts were within normal range and not significantly different from that of the controls $p > 0.05$. Platelets count was strongly and significantly correlated with alkaline phosphatase r 0.98 p 0.0001. **CONCLUSION**: There is a high prevalence of HBV serological markers among seropositive prospective blood donors and it might be associated with hepatic dysfunction. Public health education, vaccination and institution of a HBV policy on blood donor screening is advised.

Key Words: Hepatitis B virus, blood donors, liver enzymes and blood cell counts

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INTRODUCTION

Hepatitis B viral (HBV) infection is one of the common transfusion transmissible infections (TTIs) in some prospective blood donors in our center. Epidemiological and case studies have evidently documented consistently the severe pathological consequences of persistent HBV infections to include the development of chronic hepatic insufficiency, Cirrhosis and hepatocellular carcinoma.[1] Hepatitis B virus is a small DNA virus of the hepadnaviridae family of hepatotropic viruses that cause hepatitis.[2,4] It is the only hepadnavirus that causes infection in human being.[3] The virus is made up of a nucleocapsid and an outer envelope containing mainly of 3 HBsAgs that play a vital role in the diagnosis of HBV infection.[4] A marker of an acute infection is the HBsAg that is attached to the lipid membrane of the envelope.[5] Also, hepatitis B DNA, HBcAg, HBsAg, and HBeAg are also found in the blood stream.[6] The presence of this infection is first established by the presence of HBsAg in blood.[7] It is the first detectable viral antigen in most acute infections and may not be present in some chronic infections.[7&8]

There is variation of HBV infection according to geographical distributions. Sub-Saharan Africa, South East Asia, China, and the Amazon Basin are highly endemic (≥8% HBsAg sero-prevalence) or of higher intermediate endemicity (5–7.99%).[9] Countries from the Mediterranean area, Eastern Europe, the Middle East, and North-West of South America are of lower intermediate endemicity (2–4.99%).[9] Western and Northern Europe, North America, part of South America, India, and Australia have mostly low endemicity levels (<2%).[9] World Health Organization and UNICEP estimated that only 41% of Nigerians were vaccinated against HBV in 2013.[2] The risk of contracting HBV in Nigeria is very important, not just because of low vaccination but because as many as 75% of the population might be exposed.[11] In Nigeria, 13.8% prevalence rate has been reported in Lagos, 4.3% in Port Harcourt, 5.7% in Ilorin[12] and 4.2% among the blood donors in Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria.[13]

In the blood donation unit of the department of haematology and blood transfusion ABUTH, Zaria, hepatitis B sero-positive prospective blood donors (PBD) are often seen in the donor clinic. These sero-positive PBD are subsequently referred accordingly to the gastroenterology clinic. This study therefore offered the opportunity to investigate the prevalence of serologic markers of HBV infection and to assess the derangement in hepatic biochemical enzymes and some haematological parameters in HBsAg sero-positive PBD in ABUTH, Shika, Zaria, Nigeria, with a view to improving blood donor care and in the implementation and evaluation of blood donation policy criteria.

MATERIALS AND METHODS

This study was conducted at the blood donation unit of Department of Haematology ABUTH Shika in Zaria over an 11 month period from February to October 2014. Institutional ethical approval was sought and obtained from the research ethics committee. Questionnaires were administered to retrieve data and informed consent was obtained before sample collection. Haematological and Serological analyses were carried out at haematology laboratory, ABUTH, Shika, while the biochemical analysis was determined at the chemical pathology laboratory of the same

hospital. Serological hepatitis B surface markers were thereafter determined in only those that were HBV sero-positive.

Study population

Two hundred (200) family replacement blood donors (100 HBV seropositive and 100 seronegative) between the ages of 18-65 years were recruited consecutively into this study. Prospective bloodonors who are HBV sero-positive constitute the study participants while those that were sero-negative were considered as the control group. Donors were interviewed and physical examination carried out prior to enrollment. Those with high risk behavior including intravenous drugs abusers, having tatoos or below the age of 18years, or above the age of 65years or with any medical problem and those who have received HBV vaccination were excluded from the study population.

Data Collection

Haematological parameters were determined by automated haematology analyzer Mindray BC 32100, Prothrombin time was determined by manual quantitative method (LABBKITT). HBV seropositivity (Biotec) and surface markers (Eugene) were determined by rapid technique and biochemical parameters were assayed manually; ALT and AST (Randox proprietary reagents), alkaline phosphatase (King Amstrong method), total bilirubin, conjugated and unconjugated bilirubin (Maloy and Evelen method). Good laboratory research practices were adhered to in order to ensure reliable results. The manufacturer's instructions for all investigations were strictly followed according to the product manual and optimum temperature for both samples and reagents were maintained.

Statistical analysis

Results analysis was conducted with GraphPad Prism 6 statistical software, and findings presented as frequency distribution, means, standard deviation, bar charts and Student T Test to compare the subject's and control's where appropriate, p value of $\leq$ 0.05 was considered as significant.

RESULTS

Table 1: Socio-demographic characteristic of the participants

	Subjects		Controls		P-value
Variables	Frequency Mean±SD	(%)	Frequency Mean±SD	(%)	
Age	42		35		0.9056
Male	96		85		0.9999
Female	4		15		0.8675
Married	40		73		0.8233
Single	60		27		0.2048
Paracetamol ingestion	96		81		0.4326
Aspirin ingestion	4		2		0.4975
Alcohol ingestion	0		2		0.2495
Smoking Cigrates	0		5		0.4226
History of Blood Transfusion	0		0		0.4226
Vaccinated for HBV	0		0		0.4226
Multiple Sexual partners	4		20		0.7655
Having Tattoo	7		3		0.0533
Having Tribal Marks	14		9		0.2230
Having Manicure & Barbing sets	4		2		0.5676
Shared unsterilized objects	28		11		0.5535
Donated Blood before	18		99		0.4226

Table2: Results of Haematological parameters in both subjects and controls (mean ± SD)

	Hb (g/dl)	WBC ($\times 10^9$/L)	PLT ($\times 10^9$/L)	PT (Seconds)	INR
Subjects (n=100)	13.8±1.2	7.3±9.8	199±71.2	17.4±4.9	1.3±0.5
Controls (n=100)	13.9±1.5	7.2±10.7	200±77.3	14.4±1.0	1.3±0.5
P values	0.6032	0.9451	0.8976	0.0001	0.9999

Key: Hb=Haemoglobin, PCV=Packed Cells Volume, WBC=White BloodCells, PLT=Platelets, PT=Prothrombin Time and INR=International Normalized Ratio.

Table 3: Biochemical parameters in HBsAg seropositive Subjects and seronegative controls (Mean ± SD)

HBsAg Participants	Alanine Amino Transferase IU/L	Aspartate Amino Transferase (IU/L)	Alkaline Phosphatase (IU/L)	Total Bilirubin (mmol/L)	Conjugated Bilirubin (mmol/L)	Uconjugated Bilirubin (mmol/L)
Subjects (n=100)	18.6±4.0	12.8±4.0	199.2±56.0	39.9±7.4	14.0±6.0	25.9±6.4
Controls (n=100)	10.1±2.1	10.7±1.9	51.2±21.8	12.6±4.6	5.7±3.2	6.8±2.6
P values	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Table 4: Relationship between Platelets and LFTsby correlation studies (Pearson)

Alanine Amino Transferase (IU/L)	Aspartate Amino Transferase (IU/L)	Alkaline Phosphatase (IU/L)	Total Bilirubin (mmol/L)	Conjugated Bilirubin (mmol/L)	Unconjugated Bilirubin (mmol/L)
18.6±4.0	12.8±4.0	199.2±56.0	39.9±7.4	14.0±6.0	25.9±6.4
199.0±71.2	199.0±71.2	199.0±71.2	199.0±71.2	199.0±71.2	199.0±71.2
r:0.219	0.107	0.982	0.454	0.380	0.437
P:0.0250	0.2810	0.0001	0.0001	0.0001	0.0001

Key: LFTs=liver function tests, IU/L=international Unit per litre and mmol/L=mill mol per litre

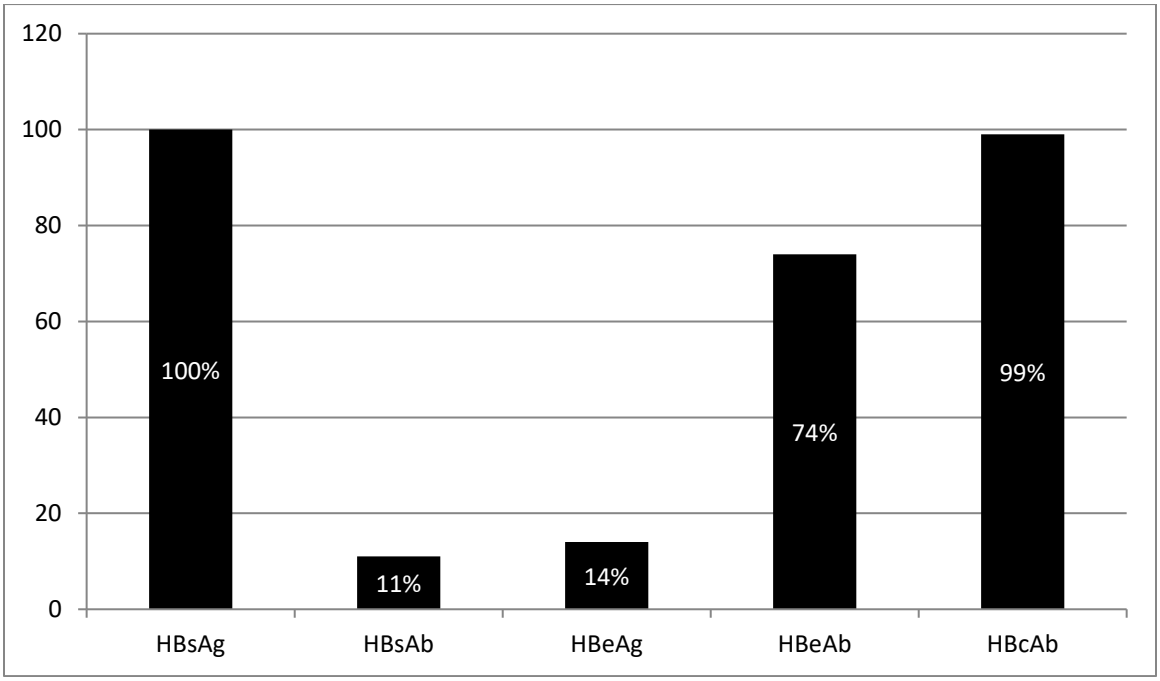

Figure 1: Prevalence of HBV Surface markers among the subjects

Key: HBV=hepatitis B virus, HBsAg=hepatitis B surface antigen, HBsAb=hepatitis B surface antibody, HBeAg=hepatitis B e antigen, HBeAb=hepatitis B e antibody, and HBcAb=hepatitis B core antibody.

DISCUSSION

Family replacement type of blood donors is the most common in our environment. This is because blood donor drive and sensitization for voluntary donation is not frequently done.

There was no significant difference in age and gender distribution between the subjects and controls. There were very few donors who are alcoholics among the participants.

ABUTH Zaria is teaching hospital and a referral center, therefore blood transfusion is frequently done, hence the need for proper screening of every blood donor. Blood donors with multiple sexual partners, especially those practicing unsafe sex are often discouraged from blood donation. Donors with tribal marks were asked on how it is practice in their culture, because traditional tribal marks are done through unsafe procedure in some cultures. This exposed individuals to contracting infections that renders them unsafe blood donors. In our blood donation centre, HBsAg detection is the major and only diagnostic screening test for HBV infection. In this study, we could not define the overall HBsAg prevalence in blood donors because we just select 100 seropositive and 100 seronegative BD among the total number of donors we had in 2014. The previous prevalence reported was 4.2% in Zaria and by most studies of blood donors during the last decade in other parts of Nigeria and oversea.[3, 12, 13&14] Although reports have stated that

75% of Nigerians are at a higher risk of contracting the virus because of low vaccination,[12]However the small sample size of this study as well as the lack of use of enzyme linked immunosorbent assay (ELISA) may be responsible for this findings. It may also be explained by the findings that some 80% of persons with chronic infection are not aware of their infection (Occult).[20] There are about 350 million chronic carriers of HBV infection in the world.[10 & 21] The prevalence ranges from 1% in some developed countries to 15% in developing countries.[15,16 & 17] About 18 million Nigerians are chronic carriers.[18] HBsAg is the first serological marker seen during HBV infection and remains the first line of HBV screening in blood donors.[2] It is advisable that donors who initially tested positive to HBsAg, be subjected to serologic confirmation using enzyme immune assay (EIA) or nucleic acid testing.[13]The use of rapid test thus serves as one of the main limitation of this study. The prevalence of HBcAb positivity in sera of blood donors positive for HBsAg was high (99%), indicating a history of infection. This is similar to the reports from medium and high-endemic areas where HBcAb prevalence in blood donors ranges between 8 and >50% (i.e., Mediterranean area, East Asia, and sub-Saharan Africa).[19] HBcAb appear in the acute phase of the infection (6-12 weeks), generally persist for the entire lifespan of an individual, and indicate HBV infection independent of the stage (acute, chronic, or recovered).[19] Individuals with only a serological HBcAb pattern can easily transmit HBV in a similar way to HBsAg reactive individuals, which correlates with the chronic carrier status of the infection.[19]

All the liver enzymes including bilirubin were elevated. Elevated levels of ALT reported in this study is in agreement with other studies where elevated ALT level in asymptomatic donor is reported to cause an unspecific marker for a wide range of active and potentially transmissible viral hepatitis infections. [21]

All the haematological parameters excluding platelets were within the normal range. This is because none of the subjects was having anaemia at the time of the study.

No correlation with LFTS except of platelet count that was strongly and significantly correlated with ALT

CONCLUSION

There is a high prevalence of HBV serological markers and elevated liver enzymes among HBV seropositive prospective blood donors and it is associated with hepatic dysfunction. Public health education, vaccination and institution of a HBV policy on blood donor screening is advised.

RECOMMENDATIONS

HBsAg infection is not uncommon in our environment, hence the need to emphasize its routine counseling and proper screening among all potential blood donors. Suitable protocol for counseling regular prospective donors on safety care, particularly as regards lifestyle and indulgencies that expose one to hepatitis B infection should be adopted. There should be a constant effort towards increasing public awareness.

We suggest that ELISA and nucleic acid testing be included in the existing blood donor screening for transfusion transmissible infections. This will improve the safety of blood transfusion. Vaccines should be given to all HBsAg seronegative blood donors and their seronegative family members after donation, so that they will be prevented subsequently.

System of proper referral and care should be established in all blood donation centres, So

that the infected prospective blood donors can access care and treatment easily. Policy on mandatory referral of all seropositive PBDs for further evaluation and treatment as appropriate

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