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Prevalence of Hepatitis B Virus among Patients Presenting with Malaria in Port Harcourt, Rivers State, Nigeria

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Abstract

The study was carried out, to determine the prevalence of Hepatitis B Virus (HBV) among patients presenting with malaria. The aim of the study is, to detect Hepatitis B Virus, using the Hepatitis B surface antigen assay among patients presenting with malaria due to the similarity in symptoms of malaria and HBV diseases. Objectives were collection of blood samples from patients presenting with malaria, who had not been previously vaccinated against HBV. Using a DIA.PRO serological kit, which is highly sensitive, to detect the surface antigen of the virus; and to provide health authorities and the public with the result gotten. The study was conducted by laboratory diagnosis by testing for the presence of HBsAg (Hepatitis B surface antigen) using the serological method. Blood specimen was collected from malaria patients (male, female, adult and children) attending the University of Port Harcourt Teaching Hospital, Choba in Port Harcourt, Rivers State, Nigeria. The blood sample was collected between the months of May and June 2015. A total of 82 blood sample was collected from the subjects through venepuncture into EDTA bottles. The samples were centrifuged and the serum from each sample was separated into another set of EDTA bottles and labelled appropriately. These blood samples were preserved by refrigerating at -20^0c. A DIA.PRO kit coated with HBAb was used for the serological analysis. A total of 27(37.93%) patients tested positive to the HBsAg. The result of the study has shown a high prevalence of HBV among patients presenting with symptoms of malaria. Suspected malaria patients, should therefore be subjected to HBV screening and necessary treatment as to reduce the spread of Hepatitis B Virus.

Keywords: Hepatitis B Virus (HBV), Malaria, HBsAg, Serology, Venepuncture

Introduction

Hepatitis B is an infectious disease caused by the hepatitis B virus (HBV) which affects the liver. It can cause both acute and chronic infections (Hepatitis B Fact

sheet, 2014). Many people are asymptomatic during the initial infection. Some develop a rapid onset of sickness with vomiting, yellow skin, fatigue, dark urine and abdominal pain. Often these symptoms last a few weeks and rarely does the initial infection result in death. It may take 30 to 180 days for symptoms to begin. In those who get infected at birth 90% develop chronic hepatitis B, while less than 10% of those infected after the age of five do (Hepatitis B FAQs for the Public-Transmission, 2011). Most of those with chronic disease are asymptomatic; however, cirrhosis and liver cancer may eventually develop. These complications results in the death of 15 to 25% of those with chronic disease.

The virus is transmitted by exposure to infectious blood or body fluids. Infection around the time of birth or from contact with other people during childhood is the most frequent method by which hepatitis B is acquired in areas where the disease is common. In areas where the disease is rare intravenous drug use and sexual intercourse are the most frequent routes of infection. Other risk factors include: working in healthcare, blood transfusions, dialysis, living with an infected person, travel to countries where the infection rate is high, and living in an institution. Tattooing and acupuncture led to a significant number of cases in the 1980s; however, this has become less common with improved sterility.

The hepatitis B viruses cannot be spread by holding hands, sharing eating utensils, kissing, hugging, coughing, and sneezing. The infection can be diagnosed 30 to 60 days after exposure. Diagnosis is typically by testing the blood for parts of the virus and for antibodies against the virus.

The infection has been preventable by vaccination since 1982. Vaccination is recommended by the World Health Organization in the first day of life if possible. Two or three more doses are required at a later time for full effect. This vaccine works about 95% of the time. About 180 countries gave the vaccine as part of national programs as of 2006. It is also recommended that all blood be tested for hepatitis B before transfusion. During an initial infection, care is based on the symptoms that a person has. In those who develop chronic disease antiviral medication such as Tenofovir or Interferon maybe useful, however these drugs are expensive. Liver transplantation is sometimes used for cirrhosis.

About a third of the world population has been infected at one point in their lives, including 240 million to 350 million who have chronic infections. Over 750,000 people die of hepatitis B each year. The disease is now only common in East Asia and sub-Saharan Africa where between 5 and 10% of adults have chronic disease. Rates in Europe and North America are less than 1%. It was originally known as serum hepatitis. Research is looking to create foods that contain HBV vaccine. The disease may affect other great apes as well.

The course of hepatitis B may be extremely variable (Zuckerman A.J, 1996). Hepatitis B virus infection has different clinical manifestations depending on the patient's age at infection and immune status, and the stage at which the disease is recognized. During the incubation phase of the disease (6 to 24 weeks), patients may feel unwell with possible nausea, vomiting, diarrhoea, anorexia and headaches. Patients may then become jaundiced although low grade fever and loss of appetite may improve. Sometimes HBV infection produces neither jaundice nor obvious symptoms (El-Serag, 2011; Zuckerman, 1996).

The asymptomatic cases can be identified by detecting biochemical or virus -specific serologic tests in their blood. They may become silent carriers of the virus and constitute a reservoir for further transmission to others.

Most adult patients recover completely from their HBV infection, but others, about 5 to 10%, will not clear the virus and will progress to become asymptomatic carriers or develop chronic hepatitis possibly resulting in cirrhosis and/or liver cancer (Zuckerman, 1996). Rarely, some patients may develop fulminant hepatitis and die. People who develop chronic hepatitis may develop significant and potentially fatal disease (Zuckerman, 1996).

In general, the frequency of clinical disease increases with age, whereas the percentage of carriers decreases. Worldwide, about 1 million deaths occur each year due to chronic forms of the disease (Bruss, 2007).

Persistent or chronic HBV infection is among the most common persistent viral infections in humans. More than 350 million people in the world today are estimated to be persistently infected with HBV. A large fraction of these are in eastern Asia and sub-Saharan Africa, where the associated complications of chronic liver disease and liver cancer are the most important health problems (Zuckerman, 1996).

A small number of long-established chronic carriers apparently terminate their active infection and become HBsAg-negative (about 2%/year).This is known as sero-convertion. Survivors of fulminant hepatitis rarely become infected persistently, and HBsAg carriers frequently have no history of recognized acute hepatitis.

The tests, called assays, for detection of hepatitis B virus infection involve serum or blood tests that detect either viral antigens (proteins produced by the virus) or antibodies produced by the host. Interpretation of these assays is complex (Bonino *et al.,* 1987).

The hepatitis B surface antigen (*HBsAg*) is most frequently used to screen for the presence of this infection. It is the first detectable viral antigen to appear during infection. However, early in an infection, this antigen may not be present and it may be undetectable later in the infection as it is being cleared by the host. The infectious virion contains an inner "core particle" enclosing viral genome. The icosahedral core particle is made up of 180 or 240 copies of core protein, alternatively known as hepatitis B core antigen, or *HBcAg*. During this 'window phase' in which the host remains infected but is successfully clearing the virus, IgM antibodies specific to the hepatitis B core antigen (*anti-HBc IgM*) may be the only serological evidence of disease. Therefore most hepatitis B diagnostic panels contain HBsAg and total anti-HBc (both IgM and IgG) (Karayiannis, 2009).

Shortly after the appearance of the HBsAg, another antigen called hepatitis B envelope antigen (*HBeAg*) will appear. Traditionally, the presence of HBeAg in a host's serum is associated with much higher rates of viral replication and enhanced infectivity; however, variants of the hepatitis B virus do not produce the 'e' antigen, so this rule does not always hold true (Liaw *et al.,* 2010). During the natural course of an infection, the HBeAg may be cleared, and antibodies to the 'e' antigen (*anti-HBe*) will arise immediately afterwards. This conversion is usually associated with a dramatic decline in viral replication.

If the host is able to clear the infection, eventually the HBsAg will become undetectable and will be followed by IgG antibodies to the hepatitis B surface antigen and core antigen (*anti-HBs* and *anti HBc IgG*) (Zuckerman, 1996). The time between the

removal of the HBsAg and the appearance of anti-HBs is called the window period. A person negative for HBsAg but positive for anti-HBs has either cleared an infection or has been vaccinated previously.

Individuals who remain HBsAg positive for at least six months are considered to be hepatitis B carriers (Lok, 2007). Carriers of the virus may have chronic hepatitis B, which would be reflected by elevated serum alanine aminotransferase (ALT) levels and inflammation of the liver, if they are in the immune clearance phase of chronic infection. Carriers who have sero converted to HBeAg negative status, in particular those who acquired the infection as adults, have very little viral multiplication and hence may be at little risk of long-term complications or of transmitting infection to others (Chu, 2007)

PCR tests have been developed to detect and measure the amount of HBV DNA, called the viral load, in clinical specimens. These tests are used to assess a person's infection status and to monitor treatment (Zoulim, 2006).

HBsAg can be detected in the serum from several weeks before onset of symptoms to months after onset. HBsAg is present in serum during acute infections and persists in chronic infections. The presence of HBsAg indicates that the person is potentially infectious (El-Serag, 2011; Takekoshi *et al* 1978; Zuckerman, 1996).

Very early in the incubation period, pre-S1 and pre-S2 antigens are present. They are never detected in the absence of HBsAg.

Anti-HBs replace HBsAg as the acute HBV infection is resolving. Anti-HBs generally persist for a lifetime in over 80% of patients and indicates immunity (El-Serag, 2011; Takekoshi *et al.,* 1978; Zuckerman, 1996).

Acute hepatitis patients who maintain a constant serum HBsAg concentration, or whose serum HBeAg persists 8 to 10 weeks after symptoms have resolved, are likely to become carriers and at risk of developing chronic liver disease (El-Serag, 2011) .

Many people with hepatitis B don't know they have it, because they are asymptomatic. The main symptoms include: Fatigue, Mild fever, Headache, Loss of appetite, Nausea, Belly pain, Diarrhea or constipation, Muscle aches and joint pain, Skin rash, Yellowish eyes and skin (jaundice). Jaundice usually appears only after other symptoms have started to go away. Most people with chronic hepatitis B are asymptomatic.

Hepatitis B Virus is spread through contact with the blood and body fluids of an infected person. It can be contracted through:

- Having sex with an infected person without using a condom.
- Sharing needles (used for injecting drugs) with an infected person.
- Getting a tattoo or piercing with tools that were not sterilized.
- Sharing personal items like razors or toothbrushes with an infected person.

A mother who has the virus can pass it to her baby during delivery. Medical experts recommend that all pregnant women get tested for hepatitis B. If they have the virus, their babies can get shots (vaccines) to help prevent infection with the virus.

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans (a type of single cell microorganism) of the *Plasmodium* type. (Malaria Fact Sheet, 2014) Malaria causes symptoms that typically include fever, fatigue, vomiting and headaches. In severe cases it can cause yellow skin, seizures, coma or death. (Caraballo, 2014)

Malaria and Hepatitis B have similar clinical and laboratory presentations. They include: Clinical presentation are fatigue, mild fever, headache, loss of appetite, nausea, joint pain and jaundice. **A**lso,the presence of IFN-gamma, IL6 and TFN alpha in the laboratory diagnosis of both infections.

Malaria continues to be a major health threat worldwide. Most regions highly endemic for malaria are also endemic for other infectious diseases, which may affect the malaria infection. (Boraschi *et al;* 2008). In this context, hepatitis B virus (HBV) infections are common in many of the malaria endemic areas. HBV is also implicated in disease severity (Schofield and Grau, 2005). In humans, results from a small investigation suggest that acute falciparum malaria modulates HBV viremia in patients with chronic HBV infection. (Brown *et al;* 1992) Moreover, a study performed in a Vietnamese hospital showed that patients with cerebral malaria had a slightly greater risk of registering positive serology for the HBV surface antigen (HBsAg); (Barcus *et al;* 2002). There is no clear evidence that the clinical status of underlying hepatitis B-related liver disease is affected during malaria infection (Barcus *et al;* 2002)

Materials and Methods

Study Area

The samples were collected from the University of Port Harcourt Teaching Hospital, Choba in Port Harcourt, Rivers State, Nigeria during the month of May and June 2015 from patients presenting with malaria.

Sample Collection

A total of 82 blood sample from malaria patients (male and female, adult and children) were collected through venepuncture into EDTA bottles (to prevent coagulation) from the University of Port Harcourt Teaching Hospital, Choba. The samples were centrifuged and the serum was separated into another set of EDTA bottles and labelled appropriately. These blood samples were preserved by refrigerating at -20^0c. These malaria patients have not been previously vaccinated for hepatitis B virus.

Materials

A DIA.PRO kit for HBsAb was used which contains the following;

- Micro titre plate
- Wash buffer concentrate
- Enzyme conjugate
- Chromogen/substrate (mixture of tetramethyl-benzidine and hydrogen peroxide)
- Sulphuric acid
- Specimen diluents
- Control serum
- Plate sealing foil

- Package insert

Other materials used but not included in the kit, includes;

- Calibrated micro litre pipettes (100ul and 50ul) and disposable plastic tips
- Distilled water
- Timer
- Absorbent tissue paper
- Thermostatic incubator
- Calibrated ELISA microwell reader with 450nm and with 620-630nm filters
- Calibrated ELISA microplate washer

Principle of the Test

The microtitre plates were coated with a preparation of highly purified HBsAg that captures HBsAg antibodies to the solid phase in the first incubation with sample specificity. After washing, captured antibodies are detected by an HBsAg labelled with peroxidise (HRP), which specifically binds the second available binding site of these antibodies. The enzymes specifically bound to wells by acting on the substrate/ chromogen mixture, generates an optical signal that is proportional to the amount of HBsAb in the sample and can be detected by an ELISA reader. The samples are pre-treated in the well with a specimen diluent able to block interference present in the samples.

Preparation of Wash Solution

400ml of the distilled water was dispensed into a 500ml measuring cylinder and 20ml of the buffer was added to the 400ml of water and then this solution was dispensed into a wash bottle to be used by the wash machine.

Quantitative Analysis

The processes involved in the quantitative analysis in this study are highlighted as follow:

- 96 strips were placed in the micro plate holder. Two wells (A_1 and B_1) were left as blanks. Then 50ul of the specimen diluents was dispensed into all the wells used except for A_1 and B_1. 100ul of different calibrators and 100ul of control serum were dispensed into the wells in duplicates. Also, 100ul of sample were dispensed into the wells from well G2. The micro plate was then incubated at 37^{o}c for 60minutes.
- The micro plate was washed 10 times using the calibrated ELISA micro plate washer to enhance an efficient result. 100ul of the enzyme conjugate was then dispensed into all wells except A_1 and B_1.
- The micro plate was then incubated for 60minutes at 37^{o}c.
- The micro plate was then washed 10 times using the ELISA micro plate washer.

- 100ul of chromogen/substrate (TMB/H_2O_2) mixture was dispensed into all the wells. Then the micro plate was incubated at room temperature (27^oc) for 20minutes.
- The enzymatic reaction was stopped by dispensing 100ul of sulphuric acid into each well (the blank wells included).
- The result was read with a micro titre plate reader at OD450 (reading), the result in A_1 and B_1 wells were blanked.

Results

Table1: Work Sheet with each box representing the wells for the calibrators and the samples of malaria patients.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL 4	S3	S17	S25	S29	S39	H5	H11	H23	H34	H41
B	BLK	CAL 4	S2	S14	S24	S30	S40	H10	H20	H22	H33	H47
C	CAL 1	CAL 5	21	S13	S23	S35	S41	H9	H19	H21	H32	H46
D	CAL 1	CAL 5	S6	S12	S22	S34	H1	H8	H18	H29	H31	H45
E	CAL 2	CS	S7	S11	S21	S33	H2	H6	H17	H28	H40	H44
F	CAL 2	CS	S8	S18	S26	S32	H3	H15	H16	H27	H38	H43
G	CAL 3	S5	S9	19	S27	S31	H7	H14	H25	H26	H37	H50
H	CAL 3	S4	S10	S20	S28	S38	H4	H12	H24	H35	H36	H49

KEY: BLK = Blank, Cal = Calibrators, CS = Control Serum, S = sample 1, H = Sample 2

Table 2: The OD450nm value gotten from the micro titre reader

	1	2	3	4	5	6	7	8	9	10	11	12
A			0.239	0.202	0.152	0.24	0.331	0.309	0.254	0.299	0.31	0.381
B			0.241	0.208	0.156	0.236	0.327	0.32	0.238	0.252	0.259	0.517
C			0.28	0.252	0.185	0.276	0.381	0.278	0.256	0.313	0.401	0.844
D			0.231	0.291	0.21	0.257	0.399	0.261	0.279	0.279	0.245	0.583
E			0.215	0.259	0.189	0.242	0.32	0.267	0.242	0.275	0.266	0.526
F			0.178	0.273	0.106	0.179	0.199	0.192	0.204	0.188	0.208	0.404
G		0.183	0.222	0.241	0.162	0.206	0.273	0.232	0.249	0.232	0.209	0.228
H		0.198	0.213	0.219	0.159	0.209	0.276	0.185	0.206	0.211	0.228	0.266

Calculation

The calculation used was according to the manufacturer's guide in order to get the cut-off point to determine the positive and negative samples.
The mean value of calibrator 2 (E1 and F1) was calculated using the OD450nm reader.
From the result sheet,
E1 = 0.317
F1 = 0.217

Therefore calibrator 2 = $\frac{0.317 + 0.217}{2}$ = $0.\frac{534}{2}$ = 0 $\frac{0.317 + 0.217}{2}$.

$0.\frac{534}{2}$

= 0.267

OD 0.267 was gotten as the cut-off point therefore:
Samples with OD450 value > mean of calibrator 2 is positive for HBsAb
Samples with OD450 value < mean of calibrator 2 is negative for HBsAb

Table3: The result of those who are positive and those who are negative using the OD0.267nm

	1	2	3	4	5	6	7	8	9	10	11	12
A			Neg ative	Neg ative	N Neg ative	Neg ative	Pos- itive	Pos- itive	Neg ative	Pos- itive	Pos- itive	Pos- itive
B			Neg ative	Neg ative	Neg ative	Neg ative	Pos- itive Pos- itive	Pos- itive	Neg ative	Neg ative	Neg ative	Pos- itive
C			Pos- itive	Neg ative	Neg ative	Pos- itive	Pos- itive	Pos- itive	Neg ative	Pos- itive	Pos- itive	Pos- itive
D			Neg ative	Pos- itive	Neg ative	Neg ative	Pos- itive	Neg ative	Pos- itive	Pos- itive	Neg ative	Pos- itive
E			Neg ative	Neg ative	Neg ative	Neg ative	Pos- itive	Pos- itive	Neg ative	Pos- itive	Neg ative	Pos- itive
F			Neg ative	Neg ative	Neg ative	Neg ative	Neg ative	Neg ative	Neg ative	Neg ative	Neg ative	Pos- itive
G		Neg- ative	Neg ative	Neg Neg ativ Neg ative	Neg ative	Neg ative	Pos- itive	Neg ative	Neg ative	Neg ative	Neg ative	Neg ativ e
H		Neg- ative	Njjj ghn bfhg Neg ative	Neg ative	Neg ative	Neg ative	Pos- itive	Neg ative	Neg ative	Neg ative	Neg ative	Neg ativ e

Percentage for negative and positive

Total samples tested= 82

Total positive= 27

Total negative= 55

Percentage positive $= \frac{27}{82}$ x 100 $= \frac{27}{82}$ = 33%

Percentage negative $\frac{55}{82}$ x 100 $\frac{55}{82}$ = 67%

Table 4: Age distribution

Age range	Total tested	Positive	Percentage nmn positive	Negative	Percentage negative
0 - 10	14	4	29	10	71
11 – 20	4	2	50	2	50
21 – 30	26	12	46	14	54
31 – 40	21	3	14	18	86
41 – 50	7	3	43	4	57
51 – 60	5	2	40	3	60
61 – 70	3	1	33	2	67
71 – 80	1	0	0	1	100
81 - 90	1	0	0	1	100

Table 5: Gender of patients

Gender	Total tested	Positive	Percentage positive	Negative	Percentage Negative
Female	58	16	28	42	72
Male	24	11	46	13	54

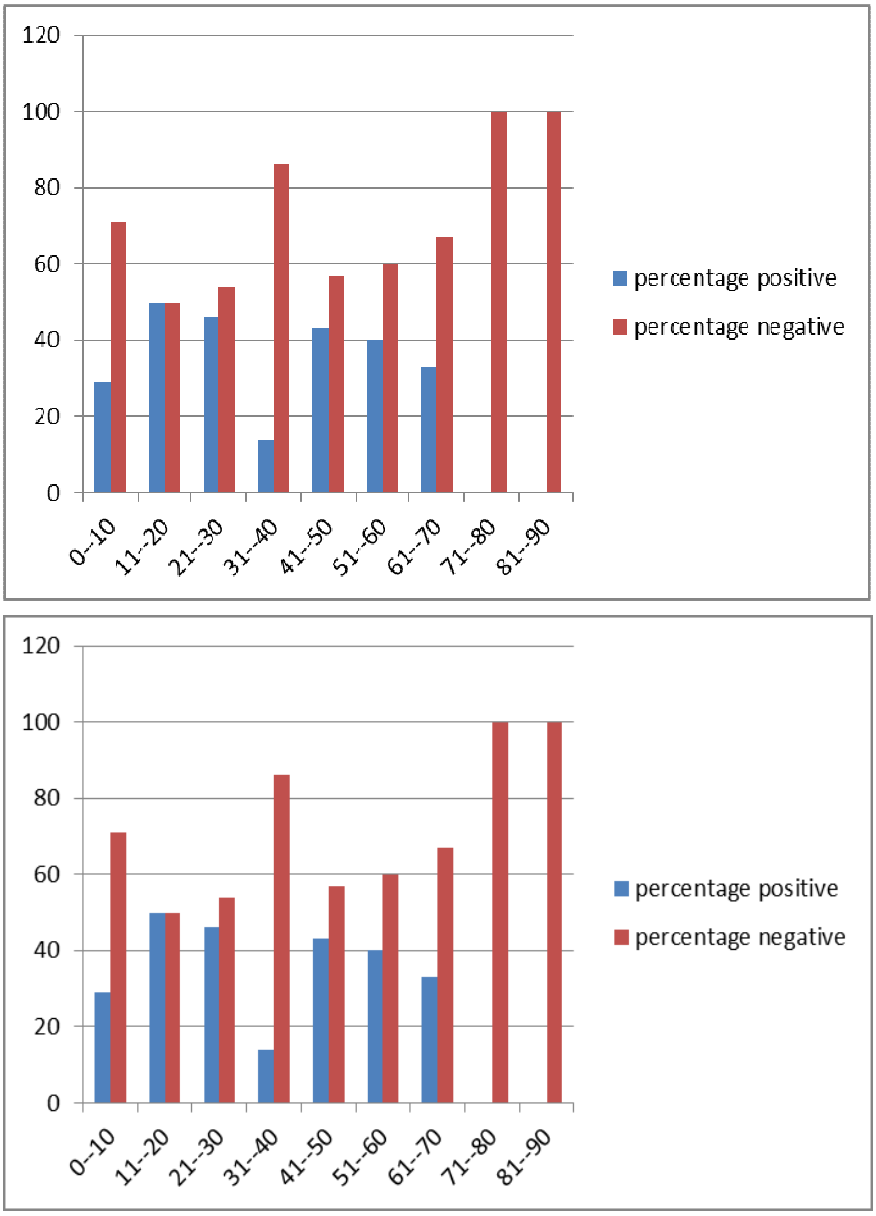

Fig. 1: Percentage age distribution

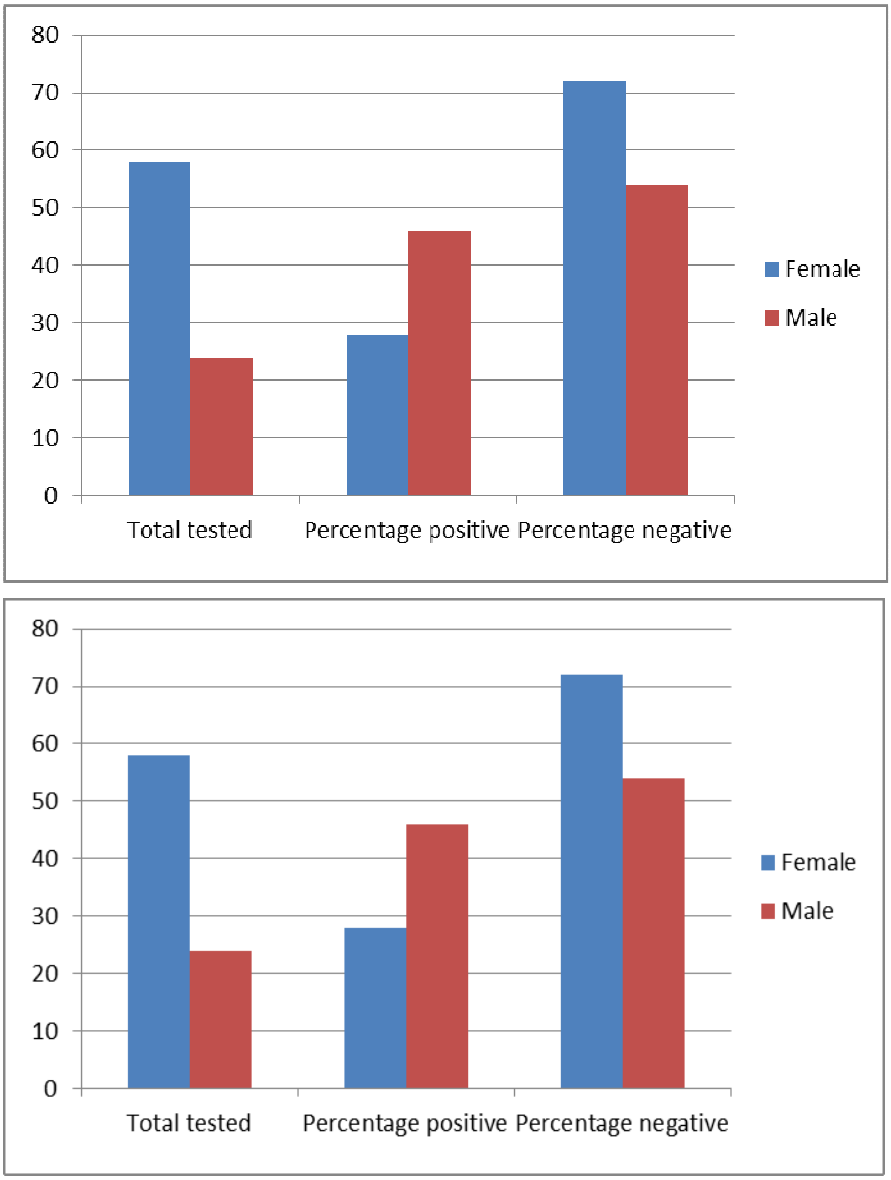

Fig. 2: Percentage gender distribution

Discussion

From the result, out of the 82 blood samples of malaria patients, 27 tested positive to HBsAg while 55 tested negative to HBsAg. Out of the 82 malaria patients tested, none had been previously vaccinated against the Hepatitis B virus. The greater occurrence of HBsAg was within age range 11-30. From this particular study, it cannot

be concluded that hepatitis B is gender bias because the gender was not evenly distributed. Out of the 82 samples, 27 samples tested positive with a percentage of 32.93% out of 100 which is a high percentage and supports Boraschi *et al;* 2008 which shows that hepatitis B is high in malaria endemic areas and can be mistaken for malaria due to the similarities in both the clinical presentations and immunological markers (Caraballo, 2014).

Hepatitis B virus is a lifelong infection except for cases of seroconversion which is rare (1 out 1000). A positive result for HBsAg could result from previous vaccination but in cases where there was no previous HBV vaccination, a positive result for HBsAg indicates the presence of HBV.

The course of hepatitis B may be extremely variable (Zuckerman A.J, 1996). Hepatitis B virus infection has different clinical manifestations depending on the patient's age at infection and immune status, and the stage at which the disease is recognized. During the incubation phase of the disease (6 to 24 weeks), patients may feel unwell with possible nausea, vomiting, diarrhoea, anorexia and headaches. Patients may then become jaundiced although low grade fever and loss of appetite may occurs. Also malaria causes symptoms that typically include fever, fatigue, vomiting and headaches. In severe cases it can cause yellow skin, seizures, coma or death. (Caraballo, 2014). **A**lso,the presence of IFN-gamma, IL6 and TFN alpha in the laboratory diagnosis of both infections

The result of this study, shows that due to the similarities in the symptoms of both malaria and HBV, and since malaria is endemic in Nigeria, most patients who are infected with HBV, mistaken it for malaria. The similarity in this two diseases have lead to wrong diagnosis among clinicians thereby giving enough time for the establishment of the virus in the patients.

Conclusion

This study shows that hepatitis B can affect any age group once the person is exposed to hepatitis B virus. Age range is one of the pointers of hepatitis B spreading and from this study the age range with the highest hepatitis B occurrence is between 11 to 30 years. This is because in this age range, individuals are sexually active and have multiple sex partners. In older adults (not sexually active), hepatitis B infection could be from sharing unsterilized sharp objects.

Since people are more enlightened on HIV than HBV, the result of this study is to be made available to the ministry of health and the public in general, thereby creating an awareness on HBV and the symptoms related to it with their similarities to malaria. Also, that patients presenting with malaria symptoms be made to undergo HBV screening in order to detect the disease early if present.

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