PREVALENCE OF HEPATITIS B SURFACE ANTIGEN AMONG BLOOD DONORS IN EKITI STATE, NIGERIA

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CERTIFICATION PAGE

This is to certify that this project work was carried out by Familoni Oluwatosin Toyin from Department of Microbiology, Faculty of Science, Federal University Oye Ekiti under my supervision and it is a fair reflection of the student's input.

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DEDICATION

This work is dedicated to the Almighty God the giver of knowledge and life for his endless grace and mercies over my life.

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I sincerely express my gratitude to God Almighty, who gave me, life, good health, sound mind, finance and literary ability to carry out this research work.

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I bow with my cap raised high to salute the scholars and authors whose ideas have been gathered together in this research work. Thank you.

Familoni Oluwatosin Oluwatoyin.

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ABSTRACT

Hepatitis B is an infectious disease caused by the Hepatitis B virus (HBV) which can affect the liver if untreated. Many people who are infected do not know because there are no symptoms during initial stages. The virus is transmitted by exposure to infectious blood or body fluids especially during sex and parenterally. The disease is prevalent in Africa especially in Nigeria. The prevalence rate is not known in Ekiti State therefore this work was carried out to provide information on the percentage of individuals suffering from Hepatitis B in Ekiti State using plasma samples of voluntary blood donors. Twenty eight blood samples were collected randomly from individual donors. The Global HBsAg immunoassay test strips manufactured in U.S.A was used for the screening. The results obtained showed 3 out of the 28 tested samples were positive giving the prevalence rate of 11%, an indication that the infection is not endemic in Ekiti state.

CHAPTER ONE

1.0 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. Many people have no symptoms during the initial infection. Some develop a rapid onset of sickness with vomiting, yellowish skin, feeling tired, 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 around the time of birth 90% develop chronic Hepatitis B while less than 10% of those infected at the age of five do. (Fontana, 2012)

Most of those with chronic disease have no symptoms: however, cirrhosis and liver cancer may eventually develop. These complications result in the death of 15 to 25% of those with chronic disease. (Fontana, 2012)

The virus is transmitted by exposure to infectious blood or body fluids. Infection around the time of birth or from contact with other people's blood 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. (Andruili, 2012). Other risk factors include working in healthcare, blood transfusion, dialysis,

living with an infected person, and travel in countries where the infection rate is high. Tattooing and acupuncture led to a significant number of cases in the 1980s, however this has become less common with improved sterility.

The virus cannot be spread by holding hands, sharing eating utensils, kissing hugging, coughing, sneezing or breastfeeding. The infection can be diagnosed 30 to 60 days after exposure. (Ijeoma, 2009). 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. 2 or 3 more doses are required at a later times for full effect. The vaccine works about 95% of the time. It is also recommended that all blood be tested for Hepatitis B before transfusion and condoms be used to prevent infections.

About a third of the world population has been infected at one point in their lives, including 240 to 350 million who have chronic infections. Over 750,000 people die of Hepatitis B each year. (Fontana, 2012). About 300,000 of these end up in liver cancer. 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%. The disease may affect other great apes as well. (Ikefuna 2009).

1.1 DESCRIPTION OF THE VIRUS

Hepatitis B Virus, is a species of the genus orthohepadnavirus, which is likewise a part of the hepadnaviridae family of viruses. This virus causes the disease hepatitis B. (Tekena, 2008)

The hepatitis B virus is classified as the type species of the orthohepadnavirus, which contains three other species: the Ground squirrel hepatitis virus, Woodchuck hepatitis virus, Woolly monkey hepatitis B virus. Viruses similar to hepatitis B have been found in all apes (orangutan, gibbons, gorillas and chimpanzees), in old world monkeys, and in a new world woolly monkeys suggesting an ancient origin for this virus in primates.

The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on its envelope proteins, and into eight genotypes (A-H) according to overall nucleotide sequence variation of the genome. The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus. Differences between genotypes affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination.

1.2 MORPHOLOGY OF HBV

The virus particles called Dane particles consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity similar to retroviruses. (Klavens 2010). The outer envelope contains embedded proteins which are involved in viral binding of, and entry into, susceptible cells. The virus is one of the smallest enveloped animal viruses with a virion diameter of of 42 nm, but pleomorphic form exist, including filamentous and spherical bodies lacking a core. These particles are not infectious and are composed of the lipid and protein that form part of the surface of the virion, which is called the surface antigen (HBsAg), and is produced in excess during the life cycle of the virus.

The virus consists of the underlisted components

- HBsAg
- HBcAg (HBeAg is a splice variant)
- Hepatitis B virus DNA polymerase
- HBx. The function of this protein is not yet well known but evidence suggests it plays a part in Hepatitis D virus requiring HBV envelope particles to become virulent.

1.3 GENOME OF HBV

The genome of HBV is made of circular DNA, but it is unusual because the DNA is not fully double-stranded. One end of the full length strand is linked to the viral DNA polymerase. The genome is 3020-3320 nucleotides long (for the full length strand) and 1700-2800 nucleotides long (for the short length strand). (Thomas 2010).

1.4 GENOTYPES

There are eight known genotypes labelled A through H. A possible new "I" genotype has been described, but acceptance of this notion is not universal. Two further genotypes are also recognized. There are at least 24 subtypes and different genotypes respond to treatment in different ways.

Type A is prevalent in Europe, Africa and South –East Asia, including the Philippines.

Type B and C are predominant in Asia

Type D is common in the Mediterranean area, the Middle East and India

Type E is localized in sub-Saharan Africa

Type F is restricted to Central and South America

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 KEY POINTS

There are about 350 million carriers of hepatitis B virus worldwide. Most of them are unaware they are carriers.

People who carry the virus often have no symptoms.

The hepatitis B virus is spread through unsafe injection practices and needle stick injuries.

The younger a person is when infected, the less likely it is that symptoms will occur. But it is more likely that he or she will become a carrier of the disease.

Most infants born to mothers who are carriers are at risk of being infected.

All children should receive hepatitis B vaccine at birth or at the age of four to six weeks, when the first visit to a clinic takes place.

A chronic carrier is more likely to develop severe chronic liver disease or liver cancer later in life. (WHO)

2.2 SIGNS AND SYMPTOMS

- Acute infection with Hepatitis B Virus is associated with acute viral Hepatitis- an illness that begins with general ill-health, loss of appetite, nausea, vomiting, body aches, mild fever and dark urine and progresses to development of jaundice. The illness last for a few weeks and then gradually improves in most affected people. The infection may be entirely asymptomatic and go unrecognized.

- Chronic infection with Hepatitis B Virus either may be asymptomatic or may be associated with a chronic inflammation of the liver (chronic Hepatitis) leading to cirrhosis over a period of several years. This type of infection dramatically increase the incidence of liver cancer. Across Europe Hepatitis B cause approximately 50% of liver cancer. Chronic carriers are encouraged to avoid consuming alcohol as it increases the risk of cirrhosis and liver cancer.

Symptoms outside of the liver are present in 1-10% of HBV-infected people and include serum sickness-like syndrome, acute necrotizing vasculitis, and membranous glomerulonephritis. About 30% of people with acute necrotizing vasculitis are HBV carriers. (Ola 2009).

2.3 EPIDEMIOLOGY

Epidemiology is the branch of science that studies the causes, patterns, effects and prevention of a particular disease. In 2004 an estimated 350 million individuals were infected worldwide. (Vitaly 2008).

2.4 TRANSMISSION

Transmission of HBV result from exposure to infectious blood or body fluids. It is 50 to 100 times more infectious than HIV. Possible forms of transmission include blood exchange during sex and transfusion with other human blood products, re-use of contaminated needles and syringes and vertical transmission from mother to child during childbirth. (Emecheke, 2009). Without intervention a mother who is positive for HBsAg has a 20% risk of passing the infection to her offspring at the time of birth. The virus can survive outside the body for 7 days. During this time, it can still cause infection if it enters the body of a person who is not protected by vaccine. (Ilechukwu 2009).

2.5 PATHOGENESIS

Hepatitis B primarily interfere with the functions of the liver by replicating in hepatocytes. There is evidence that the receptor in the closely related duck hepatitis B virus is carboxypeptidase D. (Okopi 2008). The virions are bound to the host cell via the preS domain of the viral surface antigen and are subsequently internalized by endocytosis. HBV- preS-specific receptors are expressed primarily on the hepatocyte; however, viral DNA and proteins have also been detected in extrahepatic sites, suggesting that cellular receptors for HBV may also exist on extrahepatic cells. (Olaleye 2009)

During HBV infection, the host immune response causes both hepatocellular damage and viral clearance. (Sadoh 2013). Although the innate immune response does not play a significant role in these processes, the adaptive immune response, in particular virus specific cytotoxic T lymphocytes (CTLs), contribute to most of the liver injury associated with HBV infection. CTLs eliminate HBV infection by killing infected cells and producing antiviral cytokines, which are then used to purge HBV from viable hepatocytes. Although liver damage is initiated and mediated by the CTLs, antigen-nonspecific inflammatory cells can worsen CTL-induced immunopathology, and platelets activated at the site of infection may facilitate the accumulation of CTLs in the liver.

2.6 DISEASE AND CLINICAL FEATURES

Some people develop a rapid onset of sickness with vomiting, yellowish skin, feeling tired, 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. About 5 out of 100 unvaccinated people will contract the infection sometime in their lifetime. (Damen 2008).

Not everyone with acute hepatitis has symptoms: about 7 out of 10 newly infected adults have signs or symptoms and children under 5 years of age rarely show any symptoms

Signs and symptoms of hepatitis B

· Nausea

· Lack of appetite

· Tiredness

· Muscle, joint or stomach pain

· Fever

· Diarrhea or vomiting

· Headache

· Dark urine

· Light colored stools

· yellowing of the skin and whites of the eyes (jaundice)

Complications of hepatitis B:

Having a chronic HBV can lead to serious complications, such as:

- scarring of the liver (cirrhosis): the inflammation associated with a hepatitis B infection can also lead to extensive liver scarring (cirrhosis), which may impair the liver's ability to function.

- Liver cancer: people with chronic hepatitis B infection have an increased risk of liver cancer.

- Liver failure: Acute liver failure is a condition in which the vital functions of the liver shut down. When that occurs, a liver transplant is necessary to sustain life.

- Other complications: people with chronic hepatitis B may have kidney disease, inflammation of blood vessels or anaemia.

2.7 HIGH RISK GROUPS

Certain groups of people are more at risk for complications from hepatitis B, including pregnant women, people with immune system problems, those who have not been vaccinated, people from areas of the world where hepatitis B occurs in more than 2%, those with HIV, intravenous drug users, men who have sex with men, and those who live with someone with hepatitis B. (Nor 2008).

2.8 RESERVOIR

Hepatitis B is a human disease but it has also been observed in other primates, including chimpanzees and gorillas and other great apes. (Nebia 2007).

2.9 TEMPORAL PATTERNS AND DISTRIBUTION

Hepatitis B is a serious and common infectious disease of the liver, affecting millions of people throughout the world. Three quarters of the world's population live in areas where there high levels of infection. Infection occurs very often in early childhood when it is asymptomatic and often leads to the chronic carrier state. (Alao 2009). More than two hundred million people alive today have been infected with HBV at some time in their lives. Hepatitis B prevalence is highest in sub-Saharan Africa and East Asia, where between 5-10% of the adult population I chronically infected. High rates of chronic are also found in the Amazon and the southern parts of eastern and central Europe. (WHO). In the Middle East and the

Indian subcontinent, an estimated 2-5% of the general population is chronically infected. Less than 1% of the population in Western Europe and North America is chronically infected. (Al-Moyed 2012).

2.10 PREVENTION

Vaccines for the prevention of hepatitis B have been recommended for infants since 1993. Most vaccines are given in three doses over a course of months. A protective response to the vaccine is defined as an anti-HBs antibody concentration of at least 10 Miu/ml in the recipient's serum. The vaccine is more effective in children and 95% of those vaccinated have protective levels of antibody. This drops to around 90% at 40 years of age and to around 75% in those over 60 years. The protection afforded by vaccination is long lasting even after antibody levels fall below 10 MIU/ml. Vaccination at birth is recommended for all infants of HBV infected mothers. A combination of hepatitis B immune globulin and an accelerated course of HBV vaccine prevents HBV transmission around the time of birth in 86% to 99% of cases. (Kuhrnert 2010).

All those with a risk of exposure to body fluids such as blood should be vaccinated. Testing to verify effective immunization is recommended and further doses of vaccine be given to those who are not sufficiently immunized. In females

with hepatitis B, the risk of transmission from mother to child with In Vitro Fertilization (IVF) is no different from the risk in spontaneous conception. (Wasley 2010).

In 10- to 22- year follow-up studies there were no cases of hepatitis B among those with a normal immune system who were vaccinated, only rare chronic infections have been documented.

The World Health Organization (WHO) also organizes World Hepatitis Day on July 28 every year to raise and increase awareness and understanding of viral hepatitis. (WHO).

2.11 CONTROL AND TREATMENT

Acute hepatitis B infection does not usually require treatment and most adults clear the infection spontaneously. Early antiviral treatment may be required in fewer than 1% of people, whose infection takes a very aggressive course or who are immunocompromised. On the other hand, treatment of chronic infection may be necessary to reduce risk of cirrhosis and liver cancer. (Ayliffe 2007). Chronically infected individuals with persistently elevated serum alanine aminotransferase, a marker of liver damage, and HBV DNA levels are candidates for therapy. Treatment lasts for six months to a year, depending on medication and genotype

2.12 REACTIVATION

Hepatitis B virus DNA persists in the body after infection, and in some people the disease recurs. Although rare, reactivation is seen most often following alcohol or drug use, or in people with impaired immunity. (Thabit 2012). HBV goes through cycles of replication and non-replication. Approximately 50% of overt carriers experience acute reactivation. Males with baseline ALT of 200UL/L are three times more likely to develop a reactivation than people with lower levels. Although reactivation can occur spontaneously, people who undergo chemotherapy have a higher risk.

2.13 MORTALITY RATE

An estimated 1 million people die each year from hepatitis B and its complications and approximately 2 people die each minute from hepatitis B.

2.14 LABORATORY DIAGNOSIS

The tests, called assays, for detection of hepatitis B virus infection involve serum or blood tests that detect either viral antigens produced by the virus or antibodies produced by the host. (El-sarag 2013).

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. (Hasson 2012).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 SAMPLE COLLECTION

Twenty eight blood samples were taken from random individuals from five different towns in Ekiti state namely: Ado-Ekiti, Ikere-Ekiti, Oye-Ekiti, Ifaki-Ekiti and Ido-Ekiti.

Blood samples were all obtained randomly from blood donors during blood donation. Ten millimeters (10ml) were collected from each donor. The hospital phlebotomist helped in the process of blood collection.

TABLE 1.

NAME OF TOWNS AND NUMBER OF SAMPLES COLLECTED FROM THEM:

S/N	TOWN	NUMBER OF MALE SAMPLES	NUMBER OF FEMALE SAMPLES	TOTAL
1	ADO	5	2	7
2	IKERE	4	3	7
3	OYE	1	5	6
4	IFAKI	1		1
5	IDO	4	3	7
	TOTAL	15	13	28

After collection of blood samples from individuals into EthyleneDiamineTetraA-cetic (EDTA) acid bottle the plasma was separated from blood and other component by centrifuging it at 1000rpm for 5 minutes. After separating the plasma I then emptied them into another set of EDTA bottles and making sure it wasn't containing any other component I then stored the specimen below -4^{0}C inside a freezer.

3.2 MATERIALS AND EQUIPMENT

Cotton wool, needle and syringe, EDTA bottle, gloves, centrifuge, refrigerator, ice pack, tube rack, timer, Global HBsAg test strip pack, Tourniquet, EDTA capillary tube. Twenty eight blood samples of (10ml each) were collected.

3.3 METHODOLGY

The Global HBsAg test strip is a qualitative, solid phase, two-site sandwich immunoassay for the detection of HBsAg plasma. The membrane is precoated with anti-HBsAg antibodies on the test line region of the strip. During testing, the plasma specimen reacts with the particle coated with anti-HBsAg antibodies. The mixture migrates upward on the membrane chromatographically by capillary

action to react with anti-HBsAg antibodies on the membrane and generate a colored line.

The EDTA bottle containing the plasma specimen was opened and a test strip with arrows pointing towards specimen sample was immersed into the bottle vertically for at least 15 seconds to ensure that the maximum line on the test strip when immersed in the specimen is not surpassed.

After removing the strip it was placed on a nonabsorbent flat surface started the timer the color line(s) appeared in about 30 mins.

POSITIVE: two different lines may appear. One colored line in the control region (C) and another colored line in the test region (T)

NEGATIVE: one colored line appeared in the control region (C).

INVALID: control line failed to appear.

CHAPTER FOUR

4.0 RESULTS

The following are the screening test results for 28 plasma samples from five different towns in Ekiti state for presence of hepatitis B virus. The results were collated after careful examination of the strips for at least 15 minutes.

TABLE 2.

THE PREVALENCE OF HEPATITIS B INFECTION AMONG DIFFERENT AGE GROUPS AND SEX IN EKITI STATE

S/N	SAMPLE	GENDER	AGE(YRS)	OCCUPATION	TOWN	STATUS
1	SAMPLE ONE	MALE	30	TEACHER	IKERE	NEGATIVE
2	SAMPLE TWO	MALE	1	BABY	ADO	NEGATIVE
3	SAMPLE THREE	FEMALE	28	TAILOR	OYE	NEGATIVE
4	SAMPLE FOUR	FEMALE	22	STUDENT	IKERE	NEGATIVE
5	SAMPLE FIVE	FEMALE	67	TRADER	OYE	NEGATIVE
6	SAMPLE SIX	FEMALE	30	FULL HOUSEWIFE	IDO	NEGATIVE
7	SAMPLE SEVEN	MALE	27	DRIVER	IDO	POSITIVE

TABLE 3.

THE PREVALENCE OF HEPATITIS B INFECTION AMONG DIFFERENT AGE GROUPS AND SEX IN EKITI STATE

S/N	SAMPLE	GENDER	AGE(YRS)	OCCUPATION	TOWN	STATUS
8	SAMPLE EIGHT	MALE	36	BANKER	ADO	NEGATIVE
9	SAMPLE NINE	MALE	16	STUDENT	IKERE	NEGATIVE
10	SAMPLE TEN	MALE	16	STUDENT	IKERE	NEGATIVE
11	SAMPLE ELEVEN	FEMALE	54	NURSE	IKERE	NEGATIVE
12	SAMPLE TWELVE	MALE	14	STUDENT	OYE	NEGATIVE
13	SAMPLE THIRTEEN	MALE	13	STUDENT	ADO	NEGATIVE
14	SAMPLE FOURTEEN	FEMALE	23	LABORATORY TECHNOLOGIST	IDO	NEGATIVE

TABLE 4.

THE PREVALENCE OF HEPATITIS B INFECTION AMONG DIFFERENT AGE GROUPS AND SEX IN EKITI STATE

S/N	SAMPLE	GENDER	AGE(YRS)	OCCUPATION	TOWN	STATUS
15	SAMPLE FIFTEEN	MALE	30	TEACHER	ADO	NEGATIVE
16	SAMPLE SIXTEEN	FEMALE	32	BAR ATTENDANT	ADO	NEGATIVE
17	SAMPLE SEVENTEEN	FEMALE	50	TAILOR	IKERE	NAGATIVE
18	SAMPLE EIGHTEEN	FEMALE	39	CIVIL SERVANT	OYE	NEGATIVE
19	SAMPLE NINETEEN	MALE	19	STUDENT	IFAKI	POSITIVE
20	SAMPLE TWENTY	FEMALE	4	TEEN	OYE	NEGATIVE
21	SAMPLE TWENTY ONE	FEMALE	41	TRADER	OYE	POSITIVE

TABLE 5.

THE PREVALENCE OF HEPATITIS B INFECTION AMONG DIFFERENT AGE GROUPS AND SEX IN EKITI STATE

S/N	SAMPLE	GENDER	AGE(YRS)	OCCUPATION	TOWN	STATUS
22	SAMPLE TWENTY TWO	FEMALE	17	APPRENTICE	IDO	NEGATIVE
23	SAMPLE TWENTY THREE	MALE	14	APPRENTICE	ADO	NEGATIVE
24	SAMPLE TWENTY FOUR	MALE	39	MECHANIC	IDO	NEGATIVE
25	SAMPLE TWENTY FIVE	FEMALE	44	LECTURER	ADO	NEGATIVE
26	SAMPLE TWENTY SIX	MALE	24	YOUTH CORPS	IDO	NEGATIVE
27	SAMPLE TWENTY SEVEN	MALE	40	TRADER	IDO	NEGATIVE

TABLE 6.

THE PREVALENCE OF HEPATITIS B INFECTION AMONG DIFFERENT AGE GROUPS AND SEX IN EKITI STATE

S/N	SAMPLE	GENDER	AGE(YRS)	OCCUPATION	TOWN	STATUS
28	SAMPLE TWENTY EIGHT	MALE	21	STUDENT	IKERE	NEGATIVE

Table 7.

THE NUMBER OF POSITIVE CASES OF HEPATITIS B INFECTION PER EACH TOWN

TOWN	**NUMBER OF POSITIVE CASES**
ADO-EKITI	0
IKERE-EKITI	0
IDO-EKITI	1
OYE-EKITI	1
IFAKI-EKITI	1

TOTAL NUMBER OF POSITIVE CASES=3

NUMBER OF POSITIVE MALES=2

NUMBER OF POSITIVE FEMALE=1

TOTAL POSITIVE = $^{3}/_{28}\times100=$ 11%

TOTAL NEGATIVE= $^{25}/_{28}\times100=$ 89%

POSITIVE MALES= $^{2}/_{3}\times100=$ 67%

POSITIVE FEMALE= $^{1}/_{3}\times100=$ 33%

The prevalence of the hepatitis B virus in the plasma of tested individuals in Ekiti State was 11% with the male population responsible for 67% of positive cases and female contributing 33%. Anti-HBs antibodies were detected at 3.6%frequencies only in Ido, Oye and Ifaki.

CHAPTER FIVE

5.0 DISCUSSION

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. The virus is transmitted by exposure to infectious blood or body fluids. The plasma samples collected were stored at a temperature of -4^0C since the test was not carried out instantly. The work differs from results gotten from works done in other States in 2009 as the prevalence rate is lesser than the results gotten from those states in Nigeria. (Ola, 2009).

The three positive cases are all matured individuals which implies that the infection was not gotten parentally but through exposure to infectious fluid of infected friends, colleagues or partners. The zero number of cases from Ado Ekiti and Ikere Ekiti is not surprising as the two towns are known as the two foremost towns in Ekiti and so have a lot of individuals that are health conscious living in them. Also the three positive cases were all from samples collected from the same geographic zone of the state.

5.1 CONCLUSION AND RECOMMENDATION

From this work, it can be deduced that even though hepatitis B infection is a very common disease in Nigeria, the low prevalence suggests that the hepatitis B infection is not endemic in Ekiti State. Anti-HBS antibodies were detected only in Ido, Oye and Ifaki at 3.6 prevalence rates. Prevalence was higher in males (67%) than in females (33%).

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