CHAPTER ONE
INTRODUCTION AND OBJECTIVES

1.1. 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, yellow skin, feeling tired, dark urine and abdominal pain. Often these symptoms last a few weeks. In those who get infected around the time of birth, 90% develop chronic hepatitis B, while less than 10% from those whom above 5 years develop chronic infection. 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 (Raphael and David, 2008).

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% (Raphael and David, 2008).

HBV is a hepatotropic, non-cytopathic virus and a prototype member of the family *Hepadnaviridae* with a genome size of ~3,200 base pairs. The viral genome

consists of a partially double-stranded, relaxed-circular DNA (RC-DNA), comprising a complete non coding strand (negative strand) and an incomplete coding strand (positive strand), which replicates by reverse transcription via an RNA intermediate (Maria and Narayanan, 2013).

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 and dialysis. Hepatitis B Viruse cannot be spread by holding hands, sharing eating, kissing, coughing, sneezing or breastfeeding (Raphael and David, 2008).

Hepatocellular carcinoma (HCC) is now the seventh most common cancer in men and the ninth in women, with an estimated worldwide incidence of 0.25-1.2 million new cases per year. The coastal areas of Mainland China including Hong Kong are high-risk areas with more than 25 cases per 100,000 populations per year (Chau, 2001).

Hepatocellular carcinoma, like any other cancer, develops when there is a mutation to the cellular machinery that causes the cell to replicate at a higher rate and/or results in the cell avoiding apoptosis. In particular, chronic infections with hepatitis B and/or C can aid the development of hepatocellular carcinoma

.Repeated consumption of large amounts of ethanol can have a similar effect. Besides, cirrhosis is commonly caused by alcoholism, chronic hepatitis B and chronic hepatitis C. The aflatoxin from certain *Aspergillus* species of fungus is a carcinogen and aids carcinogenesis of hepatocellular cancer by building up in the liver. The combined high prevalence of rates of aflatoxin and hepatitis B in settings like China and West Africa has led to relatively high rates of heptatocellular carcinoma in these regions (Chien *et al*., 2006).

Traditional screening regimes for the detection of HCC have included measuring serum alpha-fetoprotein (AFP) levels and performing liver ultrasounds, used together in order to improve screening accuracy, as their individual sensitivity and specificity is relatively low particularly among people with cirrhosis. (Chien *et al*., 2006).

Epidemiological observations clearly indicated that age, male sex, alcohol abuse, hepatitis B virus, and hepatitis C virus (HCV) and liver cirrhosis are the most important risk factors for developing HCC. Among all the related etiologic agents, HBV infection has the strongest association with HCC. It has been observed that there is close correlation Between the geographic distribution of HBsAg carriers and occurrence of HCC. In endemic areas such as China where the HBsAg carrier rate is more than 10%, HCC presents an incidence of up to 150 cases per 100,000 per year. On the contrary, in no endemic areas like the United States where the HBsAg carrier rate is less than 1%, HCC presents an incidence of less than 4 cases

per 100,000 per year. A prospective study from Taiwan showed that the relative risk of HCC among HBsAg-positive men was 98 as compared with HBsAg-negative men (Chau, 2001).

Although most HCCs arise in a cirrhotic liver, in HBV-related HCC tumor can frequently develop on top of chronic active hepatitis. Hepatocyte necrosis secondary to chronic HBV infection triggers an inflammatory response with the synthesis of various cytokines. Some of them, such as tumor necrosis factor, may stimulate liver-cell proliferation during which DNA mutations and chromosomal rearrangements may be produced. Furthermore, extensive fibrosis disrupts the normal lobular structure and potentially leads to a further loss of control over cell growth (Chau, 2001).

1.2. Rationale

Hepatitis B Virus infection remains a major health problem causing considerable morbidity and mortality. The World Health Organization (WHO) estimates that more than one-third of the world population has been in contact with the virus, resulting in >350 million HBV chronic carriers, with >18% of them living in Africa. Sudan is classified among the African countries with high HBV endemicity, with infection rate ranging from 6.8% in central Sudan to 26% in southern Sudan (Shaza *et al.*, 2011).

Moreover, HBV is one of the most important etiological factor for HCC in humans. It can induce HCC directly by activating cellular oncogenes or indirectly through chronic liver injury, which facilitates mutation. Screening of HBV among

patients with HCC and detection of association between them may play role in placing program to prevent hepatitis B infection which may subsequently lead to HCC.

1.3. Objectives

1.3.1. General objective

To determine Frequency of Hepatitis B Virus among hepatocellular carcinoma patients in Khartoum State.

1.3.2. Specific objectives

1. To assess specimens for the presence of hepatitis B surface antigen (HBsAg) by using ELISA assay.
2. To determine difference in infection with hepatitis B virus among hepatocellular carcinoma patients according to the gender.

CHAPTER TWO
LITERATURE REVIEW

2.1. Hepatitis B Virus

Hepatitis B Virus (HBV) is a hepadnavirus which is a 42 nm in diameter composed of partially double stranded DNA genome and 27 nm nucleocapsid core surrounded by an outer lipoproteincoat containing the surface antigen in addition to e antigen (Mason, 2008).

2.1.1. History

Blumberg won the Nobel Prize in Medicine for his discovery of the hepatitis B virus. He and his colleagues discovered the virus in 1967, developed the blood test that is used to detect the virus and invented the first hepatitis B vaccine in 1969 (Mason, 2008).

2.1.2. Classification

Hepatitis B Virus is classified as the type species of the *Orthohepadnavirus*, which contains three other species that involve *Ground squirrel hepatitis virus*, *Woodchuck hepatitis virus* and the *Woolly monkey hepatitis B virus*. The genus is classified as part of the *Hepadnaviridae* family, which contains two other genera, the *Avihepadnavirus* and a second which has yet to be assigned. 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 (Mason, 2008).

2.1.3. Structure

The virus particle 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 (Locarnini, 2004).

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 42 nm, but pleomorphic forms exist, including filamentous and spherical bodies lacking a core. These particles are not infectious and are composed of the lipid and protein that forms 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 (Howard, 1986).

2.1.4. Transmission and epidemiology

Transmission of hepatitis B virus results from exposure to infectious blood or body fluids containing blood. Possible forms of transmission include sexual contact, blood transfusions and transfusion with other human blood products re-

use of contaminated needles and syringes and vertical transmission from mother to child during childbirth. 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. This risk is as high as 90% if the mother is also positive for HBeAg (Fairly and Read, 2012).

The virus can be transmitted between family members within households, possibly by contact of non intact skin or mucous membrane with secretions or saliva containing HBV. However, at least 30% of reported hepatitis B among adults cannot be associated with an identifiable risk factor (Buddeberg *et al.,* 2008).

World Health Organization (WHO) considered HBV infection is a global public health problem. It is estimated that there are 240 million HBV carriers in the world, of who roughly 600,000 die annually from HBV-related liver disease. The implementation of effective vaccination programs in many countries has resulted in a significant decrease in the incidence of acute hepatitis B. Nevertheless, hepatitis B remains an important cause of morbidity and mortality (Raphael and David, 2008).

The prevalence of HBV carriers varies from 0.1 percent to 2 percent in low prevalence areas (United States and Canada, Western Europe, Australia and New Zealand), to 3 to 5 percent in intermediate prevalence areas (Mediterranean countries, Japan, Central Asia, Middle East, and Latin and South America), to 10

to 20 percent in high prevalence areas (southeast Asia, China, sub-Saharan Africa). A systematic review focusing on data in the United States estimated that there are 2.2 million individuals with chronic HBV, two-thirds of whom were foreign born. The wide range in HBV carrier rate in different parts of the world is largely related to differences in the age at infection, which is inversely related to the risk of chronicity. The rate of progression from acute to chronic HBV infection is approximately 90 percent for perinatally acquired infection, 20 to 50 percent for infections between the age of 1 and 5 years and less than 5 percent for adult acquired infection (Raphael and David, 2008).

2.1.5. Disease

Cause hepatitis B which is an infectious disease caused by the hepatitis B virus 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, yellow skin, feeling tired, dark urine and abdominal pain. Often these symptoms last a few weeks. In those who get infected around the time of birth 90% develop chronic hepatitis B while less than 10% of those infected after the age of five do. Most of those with chronic disease have no symptoms however; cirrhosis and liver cancer may eventually develop. These complications results in the death of 15 to 25% of those with chronic disease (Raphael and David, 2008).

2.1.6. Pathogenesis

Hepatitis B Virus primarily interferes with the functions of the liver by replicating in liver cells. The virions bind 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 hepatocytes; however, viral DNA and proteins have also been detected in extrahepatic sites suggesting that cellular receptors for HBV may also exist on extrahepatic cells. During HBV infection the host immune response causes both hepatocellular damage and viral clearance. 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) contributes 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 (Lannacone *et al.*, 2007).

2.1.7. 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 then progresses to development of jaundice. The illness lasts for a few weeks and then gradually improves in most affected people. A few people may have more severe liver disease (fulminant hepatic failure), and may die as a result (Terruault and Samael., 2005).

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 increases the incidence of hepatocellular carcinoma . Symptoms outside of the liver are present in 1–10% of HBV-infected people and include serum-sickness–like syndrome, acute necrotizing vacuities (polyarteritis nodosa), membranous glomerulonephritis, and papular acrodermatitis of childhood The serum-sickness–like syndrome occurs in the setting of acute hepatitis B, often preceding the onset of jaundice. The clinical features are fever, skin rash, and polyarteritis. The symptoms often subside shortly after the onset of jaundice (Lang, 2009).

2.1.8. Lab diagnosis

The tests, called assays for detection of HBV infection involve serum or blood that detect either viral antigens (proteins produced by the virus) or antibodies produced by the host. Interpretation of these assays is complex. 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 , alternatively known as hepatitis B core antigen (HBcAg). During this 'window' 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) (Bonino *etal.*, 1987).

Shortly after the appearance of the HBsAg, another antigen called hepatitis B e 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. 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). 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 either have cleared an infection or have been vaccinated previously. Individuals who remain HBsAg positive for at least six months are considered to be hepatitis B carriers (Lok and Mcmohan, 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).

2.1.9. 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 (fulminant hepatitis) or who are immunocompromised. On the other hand, treatment of chronic infection may be necessary to reduce the risk of cirrhosis and liver cancer (Hollinger and Lau, 2006).

Although none of the available drugs can clear the infection, they can stop the virus from replicating, thus minimizing liver damage. As from 2008, there are seven medications licensed for treatment of Hepatitis B infection in the United States antiviral drugs Lamivudine (Epivir), Adefovir (Hepsera), Tenofovir (Viread), Telbivudine (Tyzeka) and Entecavir (Baraclude), and the two immune system modulators interferon Alpha-2a and PEGylated Interferon Alpha-2a (Pegasys). The use of interferon, which requires injections daily or thrice weekly, has been substituted by long-acting PEGylated interferon, which is injected only once weekly (Albert and Caporaso, 2011).

2.1.10. Prevention

Vaccines for the prevention of hepatitis B have been routinely recommended for infants since 1991 in the United States. 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 percent of those vaccinated have protective levels of antibody (Hollinger and Lau, 2006).

2.2. Hepatocellular carcinoma

Hepatocellular carcinoma (HCC), also called malignant hepatoma is the most common type of liver cancer. Most cases of HCC are secondary to either a viral hepatitis infection (hepatitis B or C) or cirrhosis (Kumar, 2013).

2.2.1. Risk factor

The main risk factors for hepatocellular carcinoma are, alcoholism, hepatitis B hepatitis C viruses (25% of causes globally), aflatoxin, cirrhosis of the liver, hemochromatosis , wilson's disease, type 2 diabetes and hemophilia (Alter, 2007).

2.2.2. Pathogenesis

Hepatocellular carcinoma, like any other cancer, develops when there is a mutation to the cellular machinery that causes the cell to replicate at a higher rate and/or results in the cell avoiding apoptosis. In particular, chronic infections of hepatitis B and/or C can aid the development of hepatocellular carcinoma by

repeatedly causing the body's own immune system to attack the liver cells, some of which are infected by the virus, others merely bystanders. While this constant cycle of damage followed by repair can lead to mistakes during repair which in turn lead to carcinogenesis, this hypothesis is more applicable. Alternatively, repeated consumption of large amounts of ethanol can have a similar effect. Besides, cirrhosis is commonly caused by alcoholism. The aflatoxin from certain *Aspergillus* species of fungus is a carcinogen and aids carcinogenesis of hepatocellular cancer by building up in the liver (Alter, 2007).

2.2.3. Signs and symptoms

Hepatocellular carcinoma may present with yellow skin, bloating from fluid in the abdomen, easy bruising from blood clotting abnormalities, loss of appetite, weight loss, abdominal pain especially in the right upper quadrant, nausea, vomiting and feeling tired (Alter, 2007).

2.2.4. Diagnosis

Ultrasound (US) is often the first imaging and screening modality used. On US, HCC often appears as a small hypo-echoic lesion with poorly defined margins and coarse irregular internal echoes. A systemic review found that the sensitivity was 60 percent and specificity was 97 percent compared with pathologic examination of an explanted or respected liver as the reference standard. The sensitivity increases to 79% with AFP correlation .In patients with a higher suspicion of HCC (such as rising alpha-fetoprotein and des-gamma carboxyprothrombin levels), the

best method of diagnosis involves a CT scan of the abdomen using intravenous contrast agent and three-phase scanning (before contrast administration, immediately after contrast administration, and again after a delay) to increase the ability of the radiologist to detect small or subtle tumors. It is important to optimize the parameters of the CT examination, because the underlying liver disease that most HCC patients have can make the findings more difficult to appreciate. Triple phase helical CT improves the detection of these tumors (Elserage *et al.,* 2008).

2.3. Association between Hepatitis B virus and Hepatocellular carcinoma

Hepatitis B Virus (HBV) is one of the most well recognized human carcinogens. Since its discovery about 40 years ago, HBV has been studied extensively. The reduction in the incidence of childhood HCC due to mass hepatitis B vaccination in Taiwan is a dramatic demonstration of the critical etiological role of hepatitis B in HCC (Chwong and Klgoh, 2006).

2.3. Previous study

As early as 1970, chronic infection with the hepatitis B virus (HBV) was noted to be associated with the development of hepatocellular carcinoma (HCC). Subsequent studies during 1980 found that more than 80% of patients with HCC in high incidence areas, such as East Asia and sub-Saharan Africa, were seropositive for hepatitis B surface antigen (HBsAg) (Shelock *et al.,* 1980).

In Taiwan, the association of HBV and HCC is stronger in children than in adults. The rate of seropositivity for HBV nearly approached 100% in children with HCC as compared 80% in adults. Although, the incidence of childhood HCC is low worldwide, the incidence of HCC in Taiwan is relatively high and therefore, any changes in the incidence rate would be easier to detect and measure (Chang *et al* ., 1994).

Another study in which prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection was investigated in 63 Japanese patients with hepatocellular carcinoma (HCC). HBV infection was confirmed by measuring hepatitis B surface antigen and HBV-DNA in the serum, and HCV infection was confirmed by measuring antibody to HCV using a 2nd generation test and HCV-RNA in serum. Some 54.0% of the patients had HCV infection only, 27.0% had HBV infection only, and 9.5% had both HCV and HBV infection. Only 9.5% of HCC patients had neither HCV nor HBV markers. These results indicate that, in Japan, HCV and HBV infection is an important factor associated with HCC, and that the hepatitis virus may have a role in the carcinogenesis of HCC (Suga *et al*., 1994).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a cross- sectional study.

3.1.2. Study area

The study was conducted in three hospitals and centers in Khartoum State. These were Soba University Hospital, Ibrahim Malik Teaching Hospital, Ibn Sina Hospital and Antalya Diagnostic Center. The practical part of this study was done in the Research Laboratory, Sudan University of Science and Technology (SUST).

3.1.3. Study duration

This study was conducted during the period from January to May 2015.

3.1.4. Study population

Hepatocellular carcinoma patients confirmed by CT scan and ultra sound, both males and females were included.

3.2. Sample size and sampling technique

A total of seventy blood samples (n=70) were obtained from patients with hepatocellular carcinoma. Five ml of blood samples were collected.

3.3 Ethical consideration

This study was approved by the College of Medical Laboratory Sience ethical committee, SUST .

3.4. Laboratory methods

3.4.1 Collection of blood samples

A volume of 5 ml blood were collected from each patient through venipuncture technique then displaced into a plain container.

3.4.2. Sample processing

Each blood sample was centrifuged at 3000 *g* for 5 min., then serum was gently collected into epindorff tube and stored at −20°C until the serological analysis.

3.4.3. Sample analysis

The samples were analyzed for the presence of HBsAg by a commercially available enzyme -linked immunosorbent assay “HBsAg ELISA” kit (Fortress Diagnostics Limited, unit 2C Antrim technology park, Antrim, BT4I IQS United Kingdom). The assays were performed following the instructions of the manufacturer (Appendix 2). Positive and negative controls were included in each assay. According to the information included in the kit’s insert, the immunoassay used has specificity 99.94%.

3.4.4. Principle of HBsAg ELISA

The ELISA test kit provides a qualitative detection of HBsAg of hepatitis B virus in human serum or plasma, the enzyme immmunoassy based on sandwich principle in which polystyrene microtiter well strips are pre-coated with monoclonal anti HBsAg antibody. Upon addition of patient sera or plasma specific immune complex will formed that can be detected by addition antibody conjugated to enzyme Horse Reddish Peroxidase (HRP) followed by incubation at 37˚C and washing steps to remove any excess at final step chromogenic solution containing Tetra Methyle Benzendine (TMB) and urea peroxidise added to microtiter well and the enzyme HRP hydrolyze the colorless chromogenic solution to blue colored compound, which turn to yellow after addition of stop reaction solution, the intensity of the color measured reflects the amount of antigens captured in well. Wells containing samples negative for HBsAg remain colorless.

3.4.5. Procedure

All reagents and specimens were settled to reach room temperature, 20ul of specimen diluents was added to each well except the blank then 100ul of positive control, negative control and specimen were added to their respective wells. The plate was covered with plate cover and incubated for 60 minutes at 37˚C. At the end of incubation period, 50ul of HRP-conjugate was added to each well except the blank, the plate was covered and incubated for 30 minutes at 37˚C. By the end

of incubation period each well was washed 5 times with diluted wash buffer. Finally 50ul of chromogen A and chromogen B solutions were added to each well including blank, then the plate was incubated at 37˚C for 15 minutes and stop solution was added.

3.4.6. Quality control and calculation of the results

Reagent, standard and control were checked for storage, stability and preparation before starting work. Each miocroplate was considered separately when the results was calculated and interrelated; the results were calculated by relating each specimen absorbance (A) to the cut off (c.o.) of the plate.

Calculation of cut off value (C.O.) = NC × 2.1 (NC is mean of the three negative controls).

The OD value of the blank well must be less than 0.080 at 450nm.

The OD value of the positive control must be more than 0.80 at 450nm.

The OD value of the negative control must be less than 0.1 at 450 nm.

3.4.7. Interpretation of results

Positive more than cut of value.

Negative less than cut of value.

3.5. Data analysis

The data that collected from questionnaire and laboratory results were analyzed by SPSS version 15 computerized program.

CHAPTER FOUR
RESULTS

A total of seventy blood samples (n=70) were obtained from patients hospitilized with hepatocellular carcinoma in four hospitals and center in Khartoum State. The samples were collected from Soba University Hospital 8(11%), Ibrahim Malik Teaching Hospital, Ibn Sina specilized Hospital 21(31%) and Antalya Diagnostic Center 5(7%) (Table 1). The seventy samples were involve 37(52%) male and 33(48%) female as that showed in (Table 2). All specimens were examined for the presence of HBsAg using ELISA Kit. The result showed that out of 70 blood samples investigated, 18 (26%) were positive for HBsAg. The rest 52(74%) were negative (Table 3). From the positive blood samples 11(61%) were males, and 7(39%) were females (Table 4). Out of 37 male samples examined 11(30%) positive for HBsAg, while the rest 26(70%) were negative. More over out of 33 females examined 7(21%) positive for HBsAg while the rest were negative 26 (79%) (Table 5).

Table 1. Distribution of patients according to the hospital

Hospital	**Patients**	
	No.	**%**
Soba University Hospital	8	11
Ibrahim Malik Teaching Hospital	36	51
Ibn Sina Hospital	21	31
Antalya Diagnostic Center	5	**7**
Total	70	100

Table 2. Distribution of patients according to the Gender

Gender	patients	
	No.	%
Males	37	52
Females	33	48
Total	70	00

Table 3. Frequency of HBsAg among hepatocellular carcinoma patients

Results	Samples	
	No.	**%**
Positive	18	26
Negative	52	74
Total	70	100

Table 4. Frequency of HBsAg according to the Gender

Gender	Samples	
	No.	**%**
Males (no: 36)	11	61
Females (no:33)	7	39
Total	18	100

Table 5. Frequency of HBsAg in each gender separately

Result	**Gender**	**No.**	**%**
Positive (n:18)	Male	11	30
	Female	**7**	21
Negative (n:52)	Male	26	70
	Female	26	79

CHAPTER FIVE
DISCUSSION

5.1. Discussion

Since its discovery about 40 years ago, Hepatitis B Virus (HBV) has been studied extensively as causative agent of hepatocellular carcinoma. Chronic infection with HBV was noted to be associated with the development of hepatocellular carcinoma. The present study aimed at detection of HBV among hepatocellular carcinoma patients in Khartoum State. Out of 70 blood samples investigated, only 18(26%) were positive. This result is similar to that obteined in Japan by Suga *et al.*, (1994) who reported that 27% of Japanese patients with hepatocellular carcinoma were positive for HBV. But disagrees with that reported in Taiwan by Chang *et al*., (1994) who reported that over 80% of hepatocellular carcinoma patients were positive for HBV, and the rate of seropositivity for HBV was nearly 100% in children. This difference may be due to the high endimicity of Taiwan with HBV infection. In the present study males were highly infected than females with HBV (61% vs 39%). This may explained by the occupational exposure to HBV of males than females. In study carried out by Le *et al*., (2012) the result in females was (7.9%) and in males was (16.8%), and this may agree with the result obtained by this study.

5.2. Conclusion

1. There is moderate exposure to HBV infection in patients hospitalized with hepatocellular carcinoma.

2. The level of infection is higher in males than females.

5.3. Recommendations

1. Screening of the blood before transfusion.

2. extensive vaccination against HBV is recommended.

3. Further studies with large number of samples and more advanced technique are required to validate the results of the present study.

REFERENCES

1. **Albert A., and Caporaso N. (2011).** HBV therapy guidelines. *Ital J Gastroentrol.,* **43**(1): 57-63.

2. **Alter J. (2007).** Epidemiology of hepatitis B and C Viruses infection. *World J Hepatol*., **3**(12): 285-291.

3. **Bonino F., Chiaberge E., Maran C., and Piantino P. (1987).** Serologic Marker of HBV infection., *J Virol Method* **24**(2): 217-23.

4. **Buddeberg F., Schimmer B., and Spahan D. (2008).** Transfusion transmissible infections. *J Clini Anaesthio* **22**(3): 503-17.

5. **Chang M., Chen C., and Lai M. (1994).** Hepatitis B vaccination in Tawin. *N Engl Med* **336**(26): 1906-7.

6. **Chau T. (2001).** Hepatocellular Carcinoma and HBV. *Disea J Med*., **6** (2):45-8.

7. **Chien J., Hwai Y., Jun S., Chain J., and Sheng N. (2006).** Risk of Hepatocellular carcinoma. *JAMA* **29**(1):467-9.

8. **Chwong M., and Klgoh N. (2006).** Chronic HBV infection and liver cancer. *Biomed J.*, **8**(7): 345-346.

9. **Elserage H., Marrero T., Rudolph L., and Reddy R. (2008).** Diagnosis and treatment of HCC. *Engl Med World J hepatol* **7**(12): 1632-51.

10. **Fairley C., and Read T. (2012).** Vaccination against Sexually transmitted disease. *J Infec Dis*., **25**(1): 66-72.

11. **Glebe D., and Urbans S. (2007).** Viral and Cellular Determinants involved Hepadna Virus. *World J Gastero*., **5**(1): 22-38

12. **Hollinger F., and Lau D. (2006).** HBV pathway to recovery through treatment. *Clini J Gastro* **35**(4): 895-931.

13. **Howard C. (1986).** The biology of Hepadna Virus *Virol J* **67**(7): 1215-35.

14. **Karayiannis P., and Thomas C. (2009).** Encyclopedia of Human. Medical Virology. Boston Academic presses.

15. **Kumar V. (2013).** Robbins and Cotran pathologic basis of disease, Basic pathology. 7TH Ed., 467-468.

16. **Lang T. (2009).** HBV and disease. *Balti J Md*., **49**(5):18-21.

17. **Lannacone M., Sitia G., Ruggeri Z., and Guidotti L. (2007).** HBV pathogen in animal model. *J Hepatol* **46**(4): 45-47.

18. **Le V**., **Nguyen T., and Hedda H. (2012).** Prevalence of hepatitis B and C Viruses in potential blood donor in Vitnam *Indi J Med*., **136**(5) : 74-81.

19. **Locarnini S. (2004).** Molecular virology of hepatitis B virus.huttp:/ www.ncbi .nim.nih.gov/ pumped.

20. **Lok A., and Mcmahon B. (2007).** Chronic hepatitis B infection. *J Hepatol* **45**(2): 507-39.

21. **Maria J., and Narayanan K. (2013).** HBV Genetic Diversity Disease and Pathogenesis. Department of Immunology. School of Biological Science, Madurai India http:/ www.intechopen .com/books/viral replication.

22. **Mason S. (2008).** Hepadna Viridae Index of International Committee on Taxonomy of Viruses http:/ www.ncbi .gov/ICTVDB aindex

23. **Raphael R., and David S. (2008).** Rubins Pathology. Philadelphia Wolters. Lippincotts Wilkins 638-689.

24. **Shaza M., Daniel J., and Jean P. (2011).** HBV infection and recombination between HBV genotypes D and E in asymptomatic blood donors. From Khartoum state, Sudan. *J Clini Microbiol.,* **49**(1) : 298-306

25. **Suga M., Sentoa A., Arima K., Kodoma T., Lakata S., and Sakamoto H. (1994).** prevelance of HBV and HCV infection in Japanese patients with HCC. *Pubmed.*, **23**(5): 438-41.

26. **Sherlock S., Fox R., Niazia A., and Scheure P. (1980).** Chronic liver disease and primary liver cell cancer with hepatits associate antigen in serum http:/ www.literature center/pumped.

27. **Terruault N., and Samael D. (2005).** Management of the HBV in the Liver Transplantation Setting. *Euro J*., **4** (7): 16-32

28. **Zoulim F. (2006).** new nucleic acid diagnostic test in viral hepatitis http:/ www.intermedpress .com

Appendx 1