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Frequency of Hepatitis (B) Virus among Hepatocellular Carcinoma Patients in Khartoum State

تكرار فيروس الكبد الوبائي (ب) لدى المرضى المصابين بسرطان خلايا الكبد في ولاية الخرطوم

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الايه

قال الله تعالي:

(فَبَدَأَ بِأَوْعِيَتِهِمْ قَبْلَ وِعَاءِ أَخِيهِ ثُمَّ اسْتَخْرَجَهَا مِنْ وِعَاءِ أَخِيهِ ۚ كَذَٰلِكَ كِدْنَا لِيُوسُفَ ۖ مَا كَانَ لِيَأْخُذَ أَخَاهُ فِي دِينِ الْمَلِكِ إِلَّا أَنْ يَشَاءَ اللَّهُ ۚ نَرْفَعُ دَرَجَاتٍ مَنْ نَشَاءُ ۗ وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

صدق الله العظيم

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DEDICATION

To my beloved parents

To my respectful brothers and sisters

To my best friends

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Firstly, thanks to **ALLAH** who gave me the health and power to carry out thisresearch.

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ABSTRACT

Hepatitis B is infectious disease caused by hepatitis B virus (HBV) which affects the liver and may cause hepatocellular carcinoma.It is the most common type of primary liver cancer in adults, and the most common cause of death in people with liver cirrhosis It occurs in the setting of chronic liver inflammation, and is most closely linked to chronic viral hepatitis infection (hepatitis B or hepatitis C).The aim of this study was to determine the frequency of HBV among hepatocellular carcinoma patients. This Study was conducted during the period between February and May 2019.

Atotal of one hundred patients with hepatocellular carcinoma were enrolled in this study. The patients were hospitalized in Khartoum Radio Isotop Therapy Hospital. Blood sample was collected from each patient. Serum was obtained then examined for the presence of HBsAg using Enzyme Linked Immune Sorbent Assay (ELISA).

This study included patients age which is ranged from I up to 80 years. The results showed that out of 100 hepatocellular carcinoma patients (HCC) investigated71(71%) were positive for HBsAg, while 29 (29%) were negative .Regarding gender 55(55%) of studied patients were males while 45(45%) were females. high of positivity result observed among males 40 (56.3%). Regarding age patient were grouped in to four group, most of patients under study belonged to 41-60 age group, the high positivity result was observed among age group 21to 40 years 29(40.8%), while high negativity result observed among age group 41to 60 years 14 (48.3%).

In this study there was an association between age and HBV among HCC (*p.* value =0.001) The study concluded that the HBV infection in hepatocellular carcinoma patients is so high and the level of infection is higher in males than females. Further studies with large number of samples and controls group and more advanced technique are required to validate these results.

المستخلص

التهاب الكبد الفيروسي هوعبارة عن مرض معدي يسببه فيروس الكبد(ب) الذي قد يتسبب في سرطان خلايا الكبد, وهي من اكتر سرطانات الكبد التي تصيب الكبار واكثرهم مسببا للموت في الاشخاص المصابين بتليف الكبد وتحدث في حالات الاصابه بالتهاب الكبد المزمن خاصه التهاب الكبد الوبائئ (ب) .

الهدف من الدراسه هو تحديد مدي انتشار فيروس التهاب الكبد بين المرضى المصابين بسرطان خلايا الكبد في ولايهالخرطوم في الفتره بين فبرايرالي مايو 2019 .

جمعت 100 عينه من مرضى مصابين بسرطان خلايا الكبد من مستشفى الذره، جمعت عينات الدم من كل مريض ثمفصل منها المصل ، كلا العينات خضعت للفحص بحثا عن المستضد السطحي لفيروس الكبد ب باستخدام ELISA حوت الدراسه على مرضى تتفاوت اعمارهم من سنه الى 80 سنه .اظهرت النتائج انه من مجموع 100 عينه فحصت71(71%) عينه اظهرت نتائج ايجابيه بينما 29(29%) عينهاظهرت نتائج سلبيه . ومن مجموع العينات كانت 55 (55 %) عينه من الذكوربينما 45 (45 %)عينه من الاناث .تم تقسيم المرضي حسب العمر الي اربعه فئات عمريه , اعلي نتيجه ايجابيه كانت في الفئه العمريه من 21الي 40 سنه 29 (40.8%) بينما اعلي نتيجه سلبيه كانت في الفئه العمريه من 41 الي 60 سنه 14 (48.3%).

فى هذه الدراسه وجدت هنالك علاقه بين العمر والتهاب الكبد الوبائي لدى المصابين بسرطان خلايا الكبد خلصت الدراسه الي ان عدوى فيروس التهاب الكبد ب في مرض بسرطان خلايا الكبد عاليا وان النسبه في الذكوركانت اعلى منها في الاناث يوصى بدراسات اضافيه بعدد اكبرمن العينات وتقنيات متقدمه للتحقق من نتائج هذه الدراسه.

LIST OF CONTENTS

CHAPTER ONE:- INTRODUCTION

CHAPTER TWO :- LITERATURE REVIEW

CHAPTER THREE: - MATERIALS AND METHODS

CHAPTER FOUR: - RESULTS

CHAPTER FIVE: - DISCUSSION, CONCLUSION AND RECOMMENDATIONS

LIST OF TABLES

LIST OF FIGURE

LIST OF ABBREVIATIONS

AFPA	lpha Feto Protein
Anti-HBc	Anti Hepatitis B core
CT	Computed Tomography
CTL	Cyto toxic T Lymphocytes
DNA	Deoxy Ribonucleic Acid
ELISA	Enzyme Linked Immune Sorbent Assay
HBeAg	Hepatitis B Secretary Antigen
HBsAg	Hepatitis B surface Antigen
HBV	Hepatitis B virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C virus
MRI	Magnetic Resonance Imaging
NCBI	National Center of Biotechnology Information
NIH	National Institutes of health
PCR	Polymerase Chain Reaction
RNA	Rib Nucleic Acid
U/S	Ultra Sonography
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

CHAPTRE ONE

INTRODUCTION

1.1.Introduction

Hepatitis means inflammation of the liver. It is most common caused by one of the several viruses, such as hepatitis A virus, hepatitis B virus, hepatitis C virus and other viruses, toxins, bacterial infections, certain drugs, and heavy alcohol use can also cause hepatitis (Jawetz*etal*., 2007).

Hepatitis B is a silent killer disease of the liver with many carriers not realizing that they are infected with the virus (Samuel*etal*., 2004).

HBV represent global health problem worldwide. Currently, more than 350 subjects are chronically infected with HBV (El-Serag, 2012).

The infection are important risk factors for hepatocellular carcinoma and other liver related morbidity (Omer*etal*., 2001).

Exposure to the virus varied from 47%-78%. With a hepatitis B surface antigen prevalence ranging from 6.8% in central Sudan to 26% in southern sudan . Studies pointed too infection in early childhood in southern Sudan while there was a trend of increasing infection rate with increasing age in northern Sudan, HBV was the commonest cause of chronic liver disease and HCC and was the second commonest cause of acute liver failure in the Sudan (Hatim., 2008).

HBV is a hepatotropic, non-cytopathic virus and a protype member of the family hepadnaviridae with a genome size of 3,200 base pairs. The viral genome consist of a partially douple- stranded, relaxed- circular 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 blood of other people during childhoods the most frequency method by which HBV is acquired in area where the disease is common in area where disease is rare. Intravenous drug and sexual intercourse are the most frequency routes of infection, other risk factors include, working in healthcare , blood transfusion , dialysis , living with infected person , travel in countries where the infection rate is high , and living in an institution,tattoing and acupuncture lead to significant number of cases in 1980s ; however this has become less common with improved sterility. The hepatitis B virus can notbe spread

by holding hands , sharing eating utensils , kissing, hugging,coughing, sneezing, or breast feeding (Thomas, 2013).

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 (alcoholism being the most common cause of liver cirrhosis (Kumar *etal*., 2003)

HCC may directly present with yellow skin , abdominal swelling due to fluid in the abdominal cavity , easy bruising from blood clotting abnormalities , loss of appetite , unintentional weight loss , abdominal pain , nausea , vomiting , or feeling tired (Alter, 2007).

HCC like any other cancer, develop when there is a mutation to the cellular machinery 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 HCC. Repeated consumption of large amount 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(Chien*etal*., 2006).

Primary liver cancer is globally the sixth most frequent cancer (6%) and the second leading cause of death from cancer (9%) ,In 2012 it occurred in 782,000 people and in 2015 resulted in 810,500 deaths (WHO, 2014).

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 Hongkong are high- risk areas with more than 25 cases per 100,000 populations per year (Chau, 2001).

1.2. Rationale

HBV is one of the most important viruses that cause liver disease and can progress to liver cirrhosis and HCC. The infection remains a major health problem causing 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>18 % of them living in Africa. Sudan is classified among the African countries with high HBV endemicity,A high seroprevalence of HBsAg was detected in patients with liver cirrhosis ranging from 31-61% and similar carrier rates of 43-60% were found in patients with HCC.A high seroprevalence of HBsAg was detected in patients with liver cirrhosis ranging from 31-61% and similar carrier rates of 43-60% were found in patients with HCC indicating that HBV infection is perhaps the commonest and etiological risk factor for developing HCC. It induce HCC directly by activating cellular oncogenes and integration of viral genome in to infected cells can induced a non cirrhotic liver to develop HCC , or indirectly through chronic liver injury, which facilitates mutation (WHO, 2012) , so conducting this study will give information about frequency of HBV among HCC patients.

1.3.Objectives

1.3.1. General objective

To determine the frequency of HBV among hepatocellular carcinoma patients in Khartoum State.

1.3.2. Specific objectives

1 .To detect HBsAgusing ELISA technique .

2. To determine frequency of HBV among hepatocellular carcinoma patients

3. To associate between HBV and study variables (age, sex).

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO
LITERATURE REVIEW

2. Literature review

2.1.HepatitisB virus

Hepatitis B virus (HBV) is ahepadna virus which is a 42 nm in diameter composed of partially double strand DNA genome and 72 nm nucleocapsid core surrounded by an outer lipoproteincoat containing the surface antigen in addition to e antigen (Mason, 2008).

2.1.1. History

The hepatitis B virus has infected humans for at least 500 years age. The evidence was obtained from 4,500-year-old human remains. According to the 2018 study of the remains of a mummified child found in the basilica of san Domenico Maggiore concluded that the child, who had lived in the 16^{th} century, had a form of HBV, and that the virus was closely related to modern variants(Ross *etal*, 2018).

The earliest record of an epidemic caused by HBV was made by Lurman in 1885, the virus was not discovered until 1966 when Baruch Blumberg, then working at the National Institutes of Health (NIH), discovered the Australian antigen (later known to be HBsAg) in the blood of Australian people (Alter and Blumberg, 1966).

David Dane and others discovered the virus particle in 1970 by electron microscope (Dane*etal*., 1970).

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

The hepatitis B virus is classified as the type species of orthohepadnavirus , which contains three other species :the 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 asecond which has yet to be assigned , this family of virus have not been assigned to a viral order (Mason, 2008).

The virus is divided in to four major serotype (adr , adw , ayr , ayw)based on antigenic epitopes present on it is envelope protein .The viral strains have also been divided in to ten genotype (A-J)and forty sub genotypes according to overall nucleotide sequence variation of genome (Hundie*etal* ., 2017) .

differences between genotypes affect the disease severity, course and likelihood of complications , and response to treatment and possibly vaccination (Kramvis*etal*,2005)

2.1.3.Structure

The virus particle, called Dane particle (virion) , consist of anouter lipid envelop and an icosahedral nucleocapsid core composed of protein. The nucleocapsid encloses the viral DNA anda DNA polymerase that has reverse transcriptase activity similar to retrovirus(Locarnini,2004) .

The outer envelope contains embedded proteins which are involved in viral bindingand entry into susceptible cells , the virus is one of the animal viruses with a virion diameter of 42 nm , but pleomorphic forms exist, including filamentous and sphericalbodieslacking a core. These particles are not infectious and are composed of the lipid and protein that forms core of the surface of the virion, which is called the surfaceantigen (HBsAg), and is produced in excess during the life cycle of the virus(Howard,1986) .

2.1.4. Transmission

Hepatitis B virus is spread from person to person primarily by blood and blood products. Blood transfusion remains a major mode of transmission in the United StateHowever Screeing of donors has reduced the risk to 1 in 63,000 transfusion. screeing tests fail to exclude small percentage of donors who have infectious viral particle in their blood despite being negative for HBsAg. Hepatitis B virus is also found in other body fluids, including urine, bile, saliva, semen, breast milk, and vaginal secretions. It is not found in feces, however, membrane contact with any of these body fluids can result in transmission. The virus can be spread to sexual partners, and it is prevalent in homosexual men and heterosexuals with multiple partners. It can be readily spread from mother to neonate at the time of vaginal delivery- a common mode of transmission in developing countries. Intravenous drug abusers have a high incidence of hepatitis B. reuse of needles has also led to transmission of the virus during placement of tattoos and ear-piercing. Crowded enviroments such as institutions for the mentally handicapped (Frederick and Southwick, 2007).

The 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 .HBV can be transmitted between family members within households, possibly by contact of non intact skin or mucous membrane with secretion or saliva containing HBV, however at least 30%of reported hepatitis B among adults can notbe associated with an identifiable risk factor (Buddeberg*etal*., 2008).

2.1.5. HBV in Sudan

Sudan is classified among countries with a high hepatitis B surface antigen (HBsAg) Endemicity of more than 8% (WHO, 1996).

Exposure to HBV infection ranges from 47% to 78% with a hepatitis B surface antigen (HBsAg) seroprevalence ranging from as low as 6.8% in central Sudan to as high as 26% in southern Sudan (McCarthy *etal*., 1994).

Identified risk factors for HBV infection in Sudan include living in southern Sudan, parenteral antischistosomal therapy, sexual promiscuity, and scarification which is a common ritual in southern Sudan. There was no association with schistosomal infection or blood Transfusion, these rates are comparable to some African countries where seroprevalence of HBsAg was reported as rates of 15.6% in Burundi, 14% in central Africa republic, and 10% in Uganda. Lower rates were however found in other countries such as Tanzania (4.4%), Nigeria (4.98%), and Ethiopia (7%)(Raphael and David, 2008).

Seroprevalence of HBsAg among asymptomatic blood donars ranged from 12.3% in southern Sudan to 17.5% in central Sudan, These studies were carried out in the eighties and nineties when screeing of blood and blood products was only done in a few blood banks in the capital, Khartoum. In 2002, a national program for screeing blood and blood products for HBV and HCV infection was introduced throughout the whole country, A high seroprevalence of HBsAg was detected in patients with liver cirrhosis ranging from 31-61% and similar carrier rates of 43-60% were found in patients with HCC. Indicating that HBV infection is perhaps The commonest risk factor for developing HCC (Mudawi, 2008).

2.1.6. Epidemiology

HBV is a serious health problem with 2 billion people infected worldwide, and 50 million suffering from chronic HBV infection. It results in 50000- 1.2 million deaths per year. HCC accounts for 320000 deaths per year (Kumar, 2013).

The prevalence of HBV carriers varies from 0.1% to 2% in low prevalence areas (United States and Canada, Western Europe, Australia and New Zealand), to 3-5% in intermediate prevalence areas (Mediterranean countries , Japan, Central Asia, and Latin and South America),and10-20% in high prevalence areas (Southeast Asia,China, sub-Saharan Africa) Asystematic 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% for perinataly acquired infection, 20-50% for infections between the age of 1 and 5 years and less than 5% for adult acquired infection (Raphael and David, 2008).

In the United states, an estimated 1,25 million people are infected with hepatitis B, and 300,000 new cases occur annually. About 300 of these patients die of acute fulminant hepatitis, and 5% to 10% of infected patients become chronic HBV carriers (Kenneth and George, 2010).

In 2004, an estimated 350 million individuals were infected worldwide, national and regional prevalence range from over 10% in Asia to under 0.5% in the United State and northern Europe. WHO 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).

2.1.7.Risk groups for hepatitis B in developing countries

Intravenous drug abusers, homosexual men, sexual contacts of antigenic- positive persons, residents in long- stay homes for mentally handicapped people, renal dialysis patients, recipients of multiple blood products. (e.g haemophiliacs), surgeons, dentists and morticians, and infants of infectious HBsAg positive mothers (Bannister*et al*., 2006).

2.1.8. Replication of HBV

The replication of HBV is unique for several reasons, first, HBV has a distinctly defined tropism for the liver, it is small genome also necessitates economy. In addition, HBV Replicates through an RNA immediate produces and release antigenic decay particles (Murray *etal*., 2002) .

The infectious virionattach to cells and becomes uncoated. In the nucleus, the partially double strand viral genome is converted to covalently closed circular double strand DNA (cccDNA) , the cccDNA serves as template for all viral transcripts , including a 3.5-kb pre genome RNA , the pre genome RNA becomes encapsidatedwith newly synthesized HBcAg(Geo *etal*., 2010).

Within the core, the viral polymerase synthesizes by reverse transcription a negative- strand DNA copy. The polymerase starts to synthesize the positive DNA strand, but the process is not completed, cores bud from the pre- GolgiMemberane acquiring HBsAg- containing envelopes, and may exit the cell, Alternatively, cores may be reimported in to nucleus and initiate another round of replication in the same cell (Geo*etal*., 2010).

2.1.9. Disease caused by Hepatitis B virus

Hepatitis B is infectious disease caused by hepatitis B virus which affect the liver, It can cause both acute and chronic infections. Many people have no symptoms during initial

infection while some develop rapid onset of sickness with vomiting, yellow skin,feeling tired dark urine and abdominal pain , often these symptoms last a few weeks and rarely does initial infection result in death. It may take 30-180 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 after the age of live . Most of them with chronic disease have no symptoms, however cirrhosis and liver cancer may eventually develop later may result in death of 15-25% of those with chronic disease(Raphael and David, 2008).

2.1.10.Pathogenesis

After entering the blood, the virus infects the hepatocytes and viral antigens are displaced on the surface of the cells, cytotoxic Tcells mediate an immune attack against the viral antigens, inflammation and necrosis occur, immune attack against viral antigens on infected hepatocyte is mediated by cytotoxic T cells. The pathogenesis of hepatitis b is probably the result of this cell- mediated injury, because HBV itself does not cause a cytopathic effect Antigen-antibody complexes cause some of the early symptoms(arthralgias, arthritis and urticarial) and some of the complications in chronic hepatitis (e.g., glomerulonephritis, cryoglobulinemia, and vasculitis) (Levension, 2014).

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, Although liver damage is initiated and mediated by the CTLs antigen- non specific inflammatory cells can worsen CTL- induced immune pathology, and platelets activated at the site of infection may facilitate the accumulation of CTLs in the liver (Lannacone*etal*., 2007).

2.1.11. Signs and symptoms

Acute infection with HBV can be 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. It has been noted that itchy skin has been an indication as a possible symptoms of all hepatitis virustypes, the illness lasts for afew weeks and then gradually improves in most affected people. A few people may have a more severe form of liver disease known as (fulminant hepatic failure) and may die as a result.

The infection may be entirely asymptomatic and may go unrecognized (Terrault and Samuel, 2005) .

Chronic infection with HBV either may be a symptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis over a period of several years. This types of infection dramatically increases the incidence of hepatocellular carcinoma(HCC). Across Europe, hepatitis B and C cause approximately 50% of HCC, chronic carriers are encourage to avoid consuming alcohol as it increase their risk for cirrhosis and liver cancer. HBV has been linked to the development of membranous glomerulonephritis, symptoms outside the liver are present in 1-10% of HBV- infected people and include serum sickness- like syndrome, acute necrotizing vasculitis(polyarteritisnodosa), membranous glomerulonephritis, and popular acrodermatitis of childhood (Gianotti- Crostisyndrome).The serum- sickness like syndrome occur 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 but can persist throughout the duration of acute hepatitis B, other immune mediated haematological disorders, such as essential mixed cryoglobulinemia and aplastic anemia have been described as part of the extra hepatic manifestations of HBV infection, but their association is not as well- defined; therefore, they probably should not be considered etiologically linked to HBV (Liang, 2009).

2.1.12.Laboratory diagnosis

After the person infected with HBV the first virological marker detectable in serum within 1-12 weeks, usually between 8 and 12 weeks is HBsAg(Dan and Fauci, 2010).

Both acute and chronic individuals have HBs antigenaemia , the diagnosis of acute disease is confirmed by demonstrating IGM anti-HBc in the serum, this appears 2 weeks after HBsAg and disappear afew months after uncomplicated infection. IgG anti HBc persist probably life long, and is a marker of previous infection. The stage of evaluation of antigenemia and antibody production is determined by EIA tests. Viral persistence confirmed by PCR- based detection of HBV DNA in serum. Detection of HBeAg is still used as a marker of enhanced infectivity and risk of chronic liver disease(Bannister*etal*., 2006).

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 infected status and to monitor treatment, individuals with high viral load, characteristically have ground glass Hepatocytes on biopsy (Zoulim, 2006).

It based on the use of DNA fragment called the gene probe. Gene probe is relatively small, single strand DNA segment that can hunt for complementary fragment of DNA (Mumtaz*etal*., 2011).

The HBsAg test was the first available test for detect hepatitis. HBsAg appears in serum within 1 to 10 weeks after exposure. It is disappear within 4 to 6 months indicates recovery. The persistence of HBsAg beyond 6 months indicates chronic disease. The disappearance of HBsAg may be proceded by the appearance of anti-HBs, and during this period, patients may develop a serum sickness- like illness. In a large percentage of patients, anti=HBs does not rise to detectable levels for several weeks to months after the disappearance of HBsAg. During this window HBsAg and anti-HBs are both negative, and if these two tests alone are used for screeing blood donors, a small percentage of infected donors may be missed. To prevent this occurrence, blood banks also test for IgM antibody directed against HBcAg. Anti-HBs rises slowly over 6 to 12 months and usually persists for life, providing protection against re infection .Naked DNA strands and association proteins make up HBeAg. The presence of HBeAg in serum indicates active viral replication, and it persists in patients with chronic disease, it presence correlating with infectivity. As the patient with acute hepatitis B recovers, HBeAg disappears, and anti-HBe appears. Seroconversion from HBeAg to anti-HBe usually corresponds with the disappearance of hepatitis B virus DNA from the serum Quantitation of viral DNA in serum is most commonly used in the assessment of patients with chronic active hepatitis. In the patient with acute hepatitis, this test provides no significant advantages over that for HBeAg. Both tests indicate active viral replication. In patients with fulminant hepatitis, assays for HBV- DNA has been positive in the absence of other positive markers for HBV (Frederick and Southwick, 2007).

2.1.13.Treatment

Acute hepatitis B infection does not usually require treatment and most adults clear the infection spontenuosly, 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. Chronically infected individuals with persistently elevated serum alanine aminotransferase, a marker of liver damage, and HBV DNA levels are candidates for therapy (Hollinger and Lau, 2006).

Treatment lasts from six month to a year, depending on medication and genotype (Albertiand Caporaso, 2011).

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 medication 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). In 2015 the world health organization recommended tenofovir or entecavir as first- line agents .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 (Dienstag, 2008).

However some individuals are much more likely to respond than others, and this might be because of the genotype of the infecting virus or the person heredity. The treatment reduces viral replication in the liver, therapy reduces viral replication in the liver, therapy reducing the viral load (the amount of virus particles are measured in the blood (Pramoolsinsup, 2002).

2.1.14.Prevention

2.1.14.1.Passive immunization

Hence include either, purified, non infectious 22-nm spherical forms of HBsAg derived from the plasma of healthy HBsAg carriers or plasma derived vaccine, supplanted by genetically engineered vaccine derived from recombinant yeast, consisting of HBsAg particles that are non glycosylated but are otherwise in distinguishable from natural HBsAg (Weinbaum*etal*., 2009).

2.1.14.2.Pre exposure prophylaxis

It is indicated for health workers exposed to blood. Haemodialysis patients and staff, resident and staff of custodial institution for the developmentally handicapped, injection drug users, inmates of long term correctional facilities , person with multiple sexual partners , persons such as haemophiliacs who require long- term, high- volume therapy with blood derivatives, household and sexual contacts of HBsAg carriers, persons living in or travelling extensively in endemic areas, un vaccinated children under the age of 18, un vaccinated children who are immigrants from endemic countries (Weinbaum*etal*., 2009).

2.2.Hepatocellular carcinoma

Is the most common type of primary liver cancer in adults, and is the most common cause of death in people with cirrhosis (Forner*etal*., 2012).

It occurs in the setting of chronic liver inflammation, and is most closely linked to chronic viral hepatitis infection (hepatitis B or hepatitis C)or exposure to toxins such as alcohol or Aflatoxin. Certain disease, such as hemochromatosis and alpha 1- antitrypsin deficiency,

markedly increase the risk of developing HCC. Metabolic syndrome and NASH are also increasingly recognized as risk factor for HCC (Kumar, 2013).

2.2.1. Risk factors

Hepatocellular Carcinoma mostly occurs in people with cirrhosis of the liver, and so risk factors generally include factors which cause chronic liver disease that may lead to cirrhosis. Still, certain risk factors are much more highly associated with HCC than others, for example, while heavy alcohol consumption is estimated to cause 60-70% of cirrhosis , the vast majority of HCC occurs in cirrhosis associated attributed to viral hepatitis. Recognized risk factors include, chronic viral hepatitis (estimated cause of 80% of cases globally with 50% caused by chronic hepatitis B and 25% caused by hepatitis C, toxins (alcohol abuse, Aflatoxin , iron overload state as haemochromatosis (Heidelbaugh*etal*., 2006).

2.2.2.Pathogenesis

Hepatocellular Carcinoma like any other cancer develop when there are epigenetic alterations and mutations affecting the cellular machinery that cause the cell to replicate at a higher rate and /or result in the cell avoiding apoptosis (Shibata and Aburatani, 2014).

In particular chronic infection with HBV and / or HCV can aid in the development of HCC by repeatedly causing the body own immune system to attack the liver cells , some of which is infected with virus , by activated immune system (inflammatory cells) release free radicals such as reactive oxygen radicals and nitric oxide which in turns can cause DNA damage and lead to carcinogenic gene mutations, in chronic infection with HBV infection the integration of viral genome in to infected cells can directly induced a non cirrhotic liver to develop HCC , repeated consumption of large amount of ethanol can have similar effect. The toxin aflatoxin from certain aspergillus species of fungus is a carcinogen and aids carcinogenesis of HCC by building up in the liver (Alter, 2007).

2.2.3.Signs and symptoms

The classical clinical features of HCC include right upper quadrant pain and weight loss, other clinical conditions that suggest this diagnosis include worsening liver function in a patient known to have cirrhosis, acute abdominal catastrophe from rupture of a liver tumor with intra- abdominal bleeding, and some rare extra hepatic manifestation. More and more commonly though, patients are being diagnosed with HCC atan asymptomatic stage while they are being evaluated for liver transplantation or as part of routine screeing in those with cirrhosis. Symptoms at initial presentation in a series of 461 Italian patients with HCC showed approximately 23% were asyptomatic, 32% had abdominal pain, 9% malaise, 8%

jaundice, 6% anorexia, 4% weight loss, 4% haemorrhage, and 2% encephalopathy (Alfx and Adrian, 2002).

2.2.4.Diagnosis

Tumors are multifocal in the liver in 75% of cases at diagnosis. Diagnosis is usually made by history, physical examination, imaging (ultra sound, MRI or CT scan showing a liver mass consistent with HCC) and optionally elevated serum AFP (400>Ng/ml), because AFP is elevated in only 50%-75% of cases. A suspicious lesion on the sonogram generally requires additional imaging studies to confirm the stage of the tumor and sensitivity for detection of small nodules may be low. The addition of arterial phase imaging to convential CT scanning increase the number of tumor nodules detected, but in nodular cirrhotic livers the sensitivity to detect HCC is low. The overall sensitivity of MRI is similar to that of triphasic CT scan but in patients with nodular cirrhotic livers MRI has better sensitivity and specificity, confirmation of diagnosis is made by fine needle aspiration or biopsy. Elevation of AFP>400ng/mlcan be used instead of fine needle aspiration cytology for diagnosis of HCC in patients with liver cirrhosis and a focal hyper vascular liver lesion (>2cm) in at least one imaging technique. Patients with potentially resectable liver mass and AFP>400ng/ml should undergo surgery without preoperative fine needle aspiration cytology or biopsy. Any deterioration in liver function in a patient with known liver cirrhosis of any etiology should raise a suspicion of HCC (Julic and Sotiropoulos, 2010).

2.3.Association between hepatitis B virus and Hepatocellular carcinoma

Epidemiological studies have demonstrated that there is a consistent and specific causal association between HBV infection and HCC, in patient with HBV the risk of HCC was 100 times higher than in non- infected individual (Beasley *et al*., 1981).

Virologicalfactors in the pathogenesis of HCC have recently been defined. Both retrospective and prospective studies strongly supported the relation between positive HBeAg and the risk of HCC (Lin, 1991).

2.3.1. Previous studies

Sudan cancer registry reflected moderate but substantial increase over the periods 1970 to 1974 (1.7%) to 3.8% in 1979-1984 for both liver and gall bladder tumors.mohamedian studied a hundred HCC cases in 1993. Mean age was found to be 51.2 years. Sex (male: female) ratio was 4:1. The highest incidence was in the west (36%), followed by the central region (17%). In southern region, it was only 2%, All HBV markers were studied in in the HCC cases. At least 90% have one marker, 60 were positive for HBsAg, 63 were positive for anti-core and 10 were positive for anti-e. In controls, carrier rate for HBsAg was 7.4%,

Previous studies in Sudan which were done on the relationship between viral hepatitis and HCC had all established a very strong association with HBV ranging from 38.55% to 67% of HCC cases whereas HCV was implicated in 11 to 17.3% of cases (Mohamedien, 1993).

Several epidemiological studies have demonstrated significant hepatocarcinogenicity with chronic HBV infection. Hepatitis B carriers have a 10%-25% lifetime risk of developing HCC. Unlike other causes of chronic hepatitis, HBV is unique in that HCC can develop without evidence of cirrhosis (Crissien and Frenette, 2014).

Hepatitis B surface antigen (HBsAg) is not the only haematological marker that carriers a significant risk for developing of HCC. Patients with positive hepatitis B core antibody (anti-HBc) who are HBsAg-negative also remain at risk for development of HCC (Chiang *etal*., 2013).

The use of HBV vaccination has resulted in significant declines in the incidence of HCC from HBV. The East Asian neonatal vaccination program is estimated to result in a 70%=85% decrease in the incidence of hepatitis B related HCC. Despite perinatal immunization, 5%-10% of infants remain at risk of a cquiring hepatitis B infection (Wu *etal*., 2015).

HCV and HBV are the major etiological agents that lead to the development of HCC, the majority of infected people with HBV reside in the HCC high risk regions of Asia and Africa. In the Asian eastern countries, HBV is the major risk factors, and Asian countries such as Hong kong and Taiwan also had high incidence of HBV-related HCC (Asim*etal*., 2013).

In Japan HBsAg- positive cases of HCC constituted 42% in 1977-1978, but recently reduced (Kim *et al*., 2008).

In Korea approximately 65-75% of HCC patients are positive for HBsAg (Park and Kim, 2005).

In Iran, the most causes of HCC is HBV and 80% of HCC cases are positive for at least one of the markers of hepatitis B virus (Pourhoseingholi*etal*.,2010).

Anti-HCV positives are significantly associated with the development of HCC, and the co-infection of hepatitis B and C is associated with a further increased risk of HCC (Teo and Fock, 2001).

Higher rates of liver cancer occur where hepatitis B and C are common, including Asia and sub-SaharanAfrica,Males are more often affected with HCC than females (WHO, 2014)

As early as 1970, chronic infection with HBV is was noted to be associated with development of HCC subsequent studies during 1980 found that more than 80% of patient

with HCC in high incidence area, such as East Asia and sub- Saharan Africa were seropositive for hepatitis B surface antigen (HBsAg) (Sherlock*etal.*, 1980).

In Taiwan the association of HBV and HCV 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 incidence rate would be easier to detect and measure (Chang*etal.*, 1994).

The incidence of HCC is relatively lower in the western hemisphere than in eastern Asia, however, despite the statistic being low, there is an increase of HCC in the west. The diagnosis of HCC has increased since the 1980s and it is continuing to increase, making itone of the rising causeof death due to cancer. The common risk factor for HCC is HCV, along with other health issues (Goh*etal*, 2015).

Another study in which prevalence of HBV and HCV infection was investigated in 63 Japanese patients with 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 second generation test and HCV-RNA in serum. Some 54% of the patient had HCV infection only, 27% had HBV infection only, and 9.5% had both HBV and HCV 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 may have a role in the carcinogenesis of HCC (Suga*etal*.,1994).

In keeping with suggestion that HCC may be directly related to HBV infection, is the observation from several studies that elevated serum levels of HBV replication are associated with a higher risk of HCC. A recent longitudinal study of 3,653 HBsAg-positive subjects in Taiwan found that an elevated serum level of HBV DNA (more than 10,000 copies/ml ~2000 IU/ml) at baseline was a strong predictor of subsequent development of HCC, independent of serum hepatitis B e antigen (HBeAg) status, serum aminotransferases levels or the presence of cirrhosis (Chein*etal*., 2006).

HCC is one of the prevalent tumors in this country. According to WHO, the regional average value for the prevalence of HCC in Sudanese males in 1995 was between 10.8 and 20.9 per 100,000. This puts Sudan in moderately high risk areas for HCC, preceded by that of China and other higher risk countries (Hamilton and Aaltonen, 2000).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

3. MATERIALS AND METHODS

3.1.Studydesign

This wasa descriptive cross- sectional study .

3.2.Study area

This study was conducted inKhartoum Radioisotope Therapy Hospital –Khartoum.

3.3 .Study duration

The study was conducted during February to May 2019.

3.4.Study population

Patients with Hepatocellular carcinomaconfirmed by computed tomography(CT) scan and ultra sound, both males and females were included in this study.

3.5. Inclusion criteria

Patients with hepatocellular carcinoma confirmed by CT scan and ultra sound.

3.6. Exclusion criteria

Patients with cancers other than hepatocellular carcinoma and not confirmed by CT scan and ultra sound.

3.7. Sample size

One Hundred patients with hepatocellular carcinoma (n=100) were collected.

3.8. Sampling Technique

This study based on non- probabilityconveniencesampling technique.

3.9. Ethical consideration

Ethical approval to conduct this study was obtained from Faculty of Medical Laboratory sciences ofSudan University of Science and Technology (SUST), and from patients after explaining the study and it is goal.

3.10.Data collection

Data was collected using well structured- nonself interviewingquestionnaire (Appendix I).

3.11. Experimental work

3.11.1. Specimen collection

An amount of 5ml blood samples were collected from each patients using veinopuncture techniques, the available vein was located , then skin was cleaned by 70% ethanol, sterile syringe (5ml) was used to collect 5ml of blood, then the blood was dispensed in sterile plain blood container.

3.12. Specimen processing

Blood samples were centrifuged at 3000 rpm for 5-10 min , then plasma samples collected in

Toeppendof tube and stored at -20degree centigrade until serological analysis .

3.13.(ELISA)for detection of HBsAg

Samples were analayzed for presence of HBsAgusingcommercially available enzyme linked Immune sorbent assay (HBsAg ELISA) kit(Fortress Diagnostic Limited, United Kingdom).

The assay was performed following the instruction of manufacture, positive and negative controls include in theeach assay, According to information included in the kit the immune assay used has specificity 99.94%.

3.14.Procedure

All reagents and specimens were brought to room temperature(+20 to +30 degree centigrade) reserved one well for blank, 20 micron of diluent was added to each well.

Of each control and specimen (100ml) were added to appropriate wells of the microtitter plate

The reaction plate was incubate in 37°c in water bath or incubator for 60 min .at the end of incubation period , 50 micron of horse reddish peroxidase-conjugate was added to each well except the blank the plate was covered and incubate for 30 min at 37◦c .

At the end of incubation period each well was washed 5 times with diluted buffer. Finally 50micro of chromogenA and B solutions were added to each well including blank.

Then the plate was incubated at 37°c for 15min and stop solution was added.

3.15. Quality control and calculation of the results

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

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

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

3.16. Interpretation of results

Positive results were more than cut off value, and negative results were less than cut off value.

Borderline: samples with absorbance to cut-off ratio between 0.9 and 1.00 are considered borderline samples and retesting is recommended. Repeatedly positive samples can be considered positive for HBsAg.

3.17. Data analysis

The data that collected from questionnaire and laboratory results was analysed by SPSS version 16 computerized program – using Frequency-chi square and descriptive statistic tests. *P*.value of < 0.05 was considered as significant for all study results.

CHAPTER FOUR

RESULTS

CHAPTER FOUR

4. RESULTS

A total number of hundred patients with hepatocellular carcinoma were enrolled in this studyto detect hepatitis B virus. The patients were hospitalized in Khartoum Radio Isotop Therapy Hospital.

patients age ranged from 1 to 80 years with mean of 42.0 and ± 17.0 STD.

According to their age, patients are divided in to four age groups as follow :

1-20 years represent 12 (12%),21-40 years represent 32 (32%),41-60 years represent 42 (42%) and age group 61-80 years represent 14 (14%).Among total studied population 55 (55%) were males and 45 (45%) were females as in Table(4.1).

The results showed that out of totalpatients investigate, 71 (71%) were positive for HBsAg. The rest 29 (29%) were negative as shown in figure (4.1).

Out of 55 males examined 40 (56.3%) were positiveforHBsAg, while the rest 15 (51.7%) were negative. Moreover out of45 females examined 31 (43.7%)were positive for HBsAg, while 14(48.3%) were negative. The result showed statistical insignificant difference between positive HBsAgand gender *p*.value = 0.674.Table (4.3).

Most of studied patient belong to 41-60 (42 (42%)) age group followed by 21-40(32(32%)) age group and most of positive HBV was observed among 21-40 age group (29(40.8)) There was statistical significant difference between HBsAg and age.*P*. value = 0.001.Table (4.4).

Table (4.1) Frequency of HBV among HCC patients regarding their gender

Sex	Frequency	Percentage (%)
Male	55	55%
Female	45	45%
Total	100	100%

Table (4.2) Frequency of HBV among HCC patients regarding to age group

Age group	Frequency	Percentage (%)
1-20	12	12%
21-40	32	32%
41-60	42	42 %
61-80	14	14 %
Total	100	100 %

Fiqure(4. 1)ELISA results

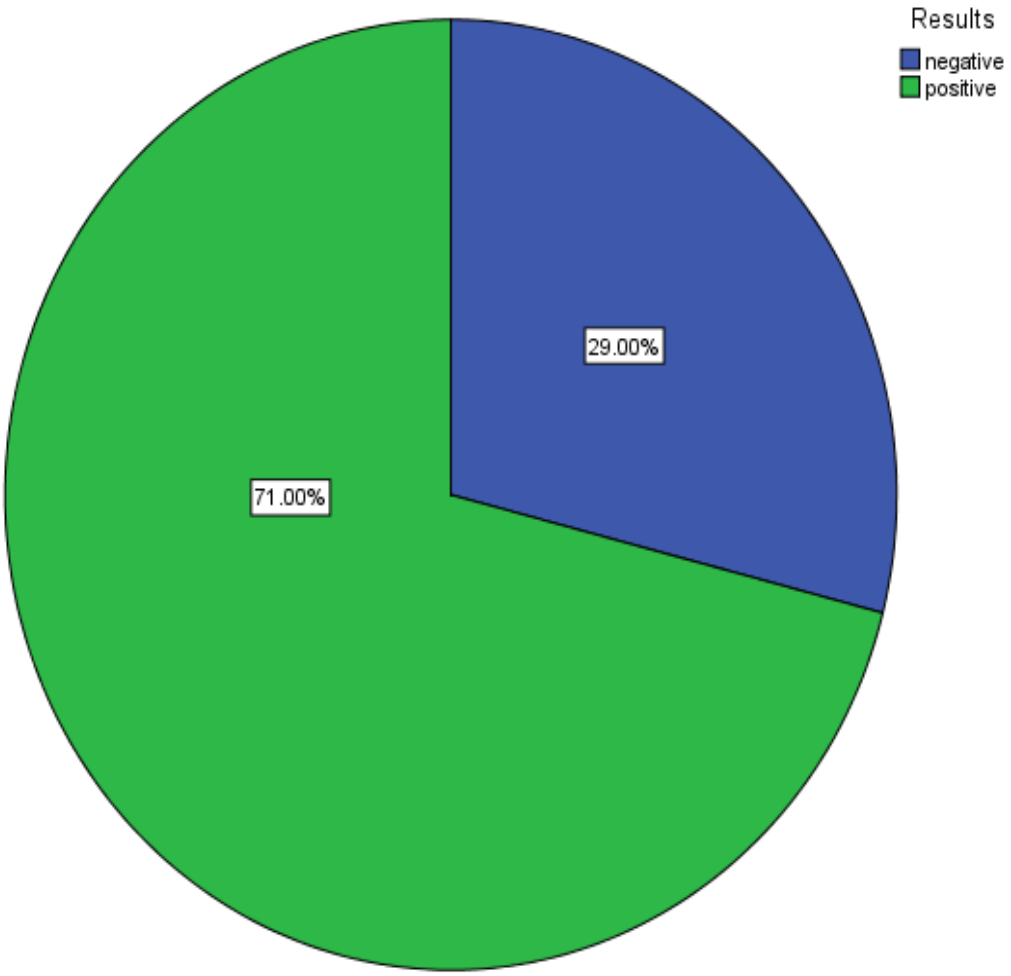

Table (4.3) Association between HBV among HCC patient and gender

Gender	Positive	Negative	Total	*P*. value
Male	40 (56.3%)	15 (51.7%)	55 (55%)	0.674
Female	31 (43.7%)	14 (48.3%)	45 (45%)	
Total	71 (100%)	29 (100%)	100 (100%)	

Table (4.4) Association of HBV among HCC patients and age

Age group	Positive	Negative	Total	*P*.value
1-20	10 (14.1%)	2 (6.9%)	12 (12%)	0.001
21-40	29 (40.8%)	3 (10.3%)	32 (32%)	
41-60	28 (39.4%)	14 (48.3%)	42 (42%)	
61-80	4 (5.6%)	10 (34.5%)	14 (14%)	
Total	71 (100%)	29 (100%)	100 (100%)	

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

CHAPTER FIVE

5.DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

HBV is a serious growing problem in African and Arab countries, and they have great affect on community and future generationhealth. So we need great effort to conduct studies that may give guidelines for proper planning to deal with the health problem that related to HBV infection, chronic infection with HBV is also noted to be associated with development of hepatocellular carcinoma (Nabil, 2005).

The present study aimed at detection of HBV among hepatocellular carcinoma patients in Khartoum state. Out of total HCC studied patients, 71 (71%) were positive, the Remaining 29 (29%) were negative, This result is similar to that obtained in Taiwan by Chang *et a*l., (1994) who reported that over 80% of HCC patients were positive for HBV , and also slightly higher than study carried out by Gogos*etal*.,(2003) who found that the prevalence of HBV markers is 20-60% and greater than 60% in high endemicity countries, But the result is higher than was that result obtained in Japan by Suga*etal*., (1994) who reported that 27% of Japanese patients with HCC were positive for HBV . This differences may be due to the low endemicity of Japan with HBV infection .

In this study females were less infected than males with HBV (45% and 55%) respectively which is slightly higher with study carried out by Le *etal*., (2012) in which the result in female was 7.9% and in males was (16.8%).

Similarly in Sudan, the Western region which could be estimated as extension of Sudan to the west both geographically and ethnically was the region with highest prevalence. Nine out of 18 western patients (50%) were positive for HBV (Hatim, 2008).

The south showed marked resistance to develop HCC in spite of the high prevalence of HBV There. HBsAgseroprevalence in Sudan varied from 7-26% among the general population. It was highest in the south . However, the exposure rate varied from 53-78%. Mother to child transmission was studied in juba, south of sudan , it was found that 55.5% of HBsAg positive mothers had children with positive serology whereas 11.4% of negative HBsAg mothers had positive children for the virus (11.4%) n=88 .The southern region shows a high risk areas of vertical transmission of HBV of 55% but in contradiction a much lower incidence of HCC compared to other region. This epidemiologic observation may explain the less vulnerability of the southern to develop HCC in spite of high prevalence of HBV in the south (Hatim, 2008).

WHO classify Sudan among the countries with HBV endemicity of >8% . HBV associated risk for the development of HCC is obvious compared to the general population (Hamilton and Aaltonen, 2000).

In Saudi Arabia, HBsAg was positive in 65% of HCC cases. In Nigeria the estimated rate in 1970 was 6.6\100,000. The most common predisposing factor was HBV(Mustapha, 2003).

Among African countries, West African countries like Nigeria and Senegal reports high prevalence rate of HCC mostly due to HBV. The prevalence of HBV infection in adult population of Senegal is up to 85%. HBV markers were detected in 59.4% of sera of children. Thisexplain the high incidence of HCC (Sall, 2004).

In a Mozambique, the prevalence of HBVsAg in HCC patients was 60% of the studied population (n=328) (Zakim, 2001).

5.2. Conclusion

The study concluded that the exposure rate of HBV among hepatocellular carcinoma patients is relatively high.

The higher percentage of HBV infection observed in the age group 21-40 years.

The level of infection is higher in males than females.

In this study there was an association of HBV among HCC and age.

5.3. Recommendations

Extensive vaccination against HBV is recommended.

Increase the Educational level about the virus, it is transmission and prevention forThepatients.

Maintenance of cancer registry in Sudan.

More health care level for HCC patients.

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

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Appendices

Appendix 1

Sudan University of Science and Technology

Collage of Graduate Studies

Department of Microbiology

Questionnaire on Frequency of HBV among Hepatocellular Carcinoma Patients in Khartoum state

ID No:

Name: ……..

Age : ……....

Age group:

1-20 years…… ☐

21-40 years …… ☐

41-60 years…….. ☐

61-80 years…….. ☐

Sex: Male ☐ **nale** ☐

Results:

+veHBsAg ☐ **_veHBsAg** ☐

Appendix 2

Principle of HBsAgELISA

The HBsAg ELISA kit provide is a fast test for the qualitative detection of the presence of HBsAg in serum or plasma (heparin, citrate or EDTA) specimen, the test utilize monoclonal and polyclonal (anti guinea pigs)antibodies to selectively detected elevated levels of HBsAg in serum or plasma

The HBsAg ELISA kits is a solid phase based on the sandwich principle, the solid phase of microtiter plate is made of poly styrene wells coated with mouse monoclonal antibodies specific for HBsAgwherease guinea pig polyclonal antibody is purified by affinity chromatography is used to prepare the anti-HBs peroxidase (horse reddish peroxidase) conjugate in the liquid phase

When the serum or plasma containing HBsAg is added to the anti- HBs antibody- coated wells together with the peroxidase conjugated anti- HBs antibody and incubated, an antibody-HBsAg- peroxidase complex will form on the wells.

after washing the microtiter plate to remove un bound material, second antibody conjugated to the enzyme HRP and directed against different epitope of HBsAg is added to the wells.

During the second incubation steps, these HRP conjugated antibodies will be bound to any anti- HBsAg complexes previously formed during the first incubation, and the unbound HRP conjugate is then removed by washing. After washing to remove unbound conjugate, chromogen solutions containing TMB and urea peroxidase are added to the wells.

In the presence of antibody- antigen- antibody HRP sandwich immune complex, the colourlesschromogens are hydrolized by the bound HRP conjugate to a blue coloured product. The blue color turns yellow colour after stopping the reaction by using the stop solution sulphuric acid, the color was read as optical density in order to determine the result of the test. Wells containing samples negative for HBsAg remain colourless.

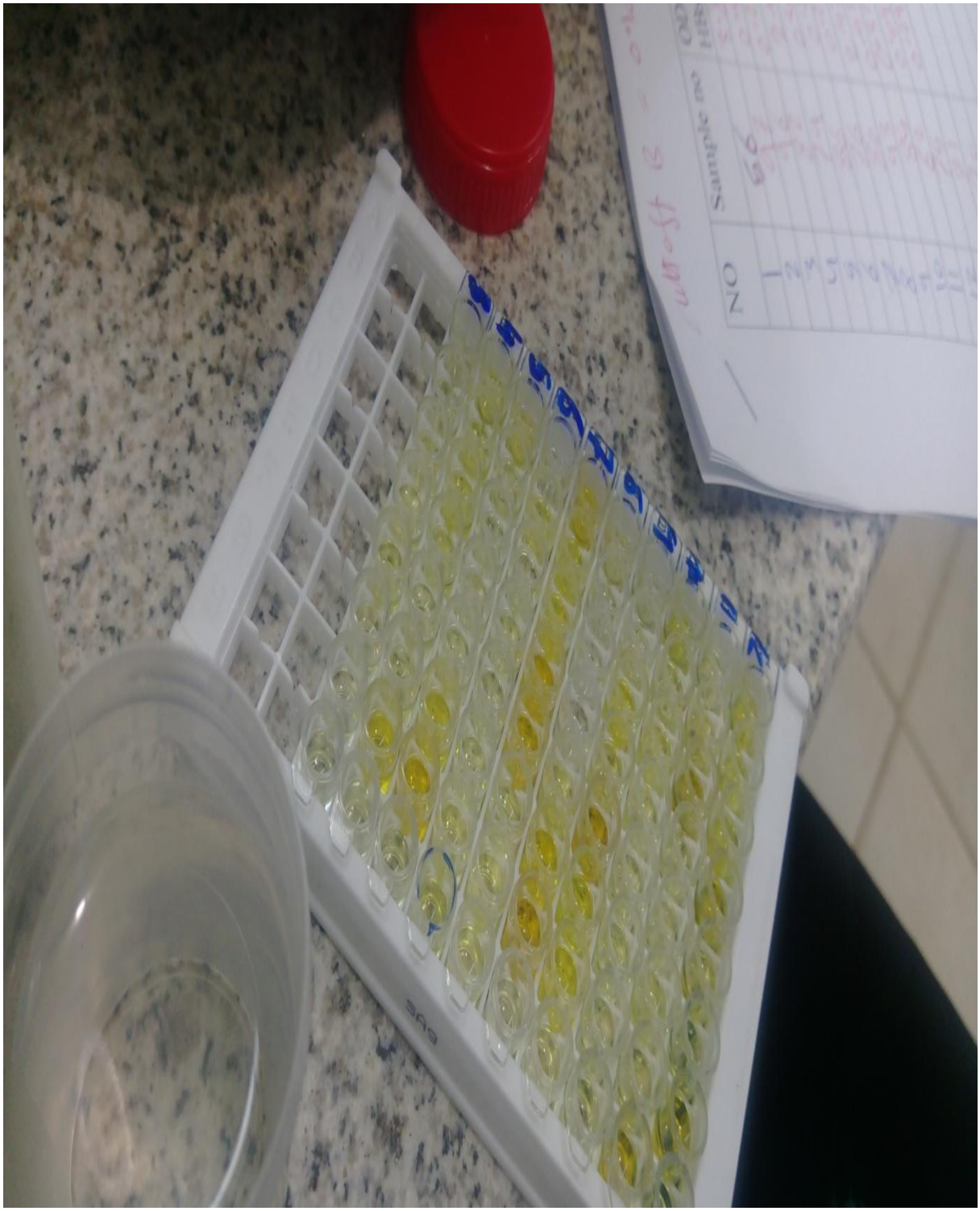
Appendix 3

Colour plate (1)ELISA Washer (Enzyme Linked Immune Sorbent Assay)

Appendix 4

Colour plate (2)ELISA Kit

Appendix 5

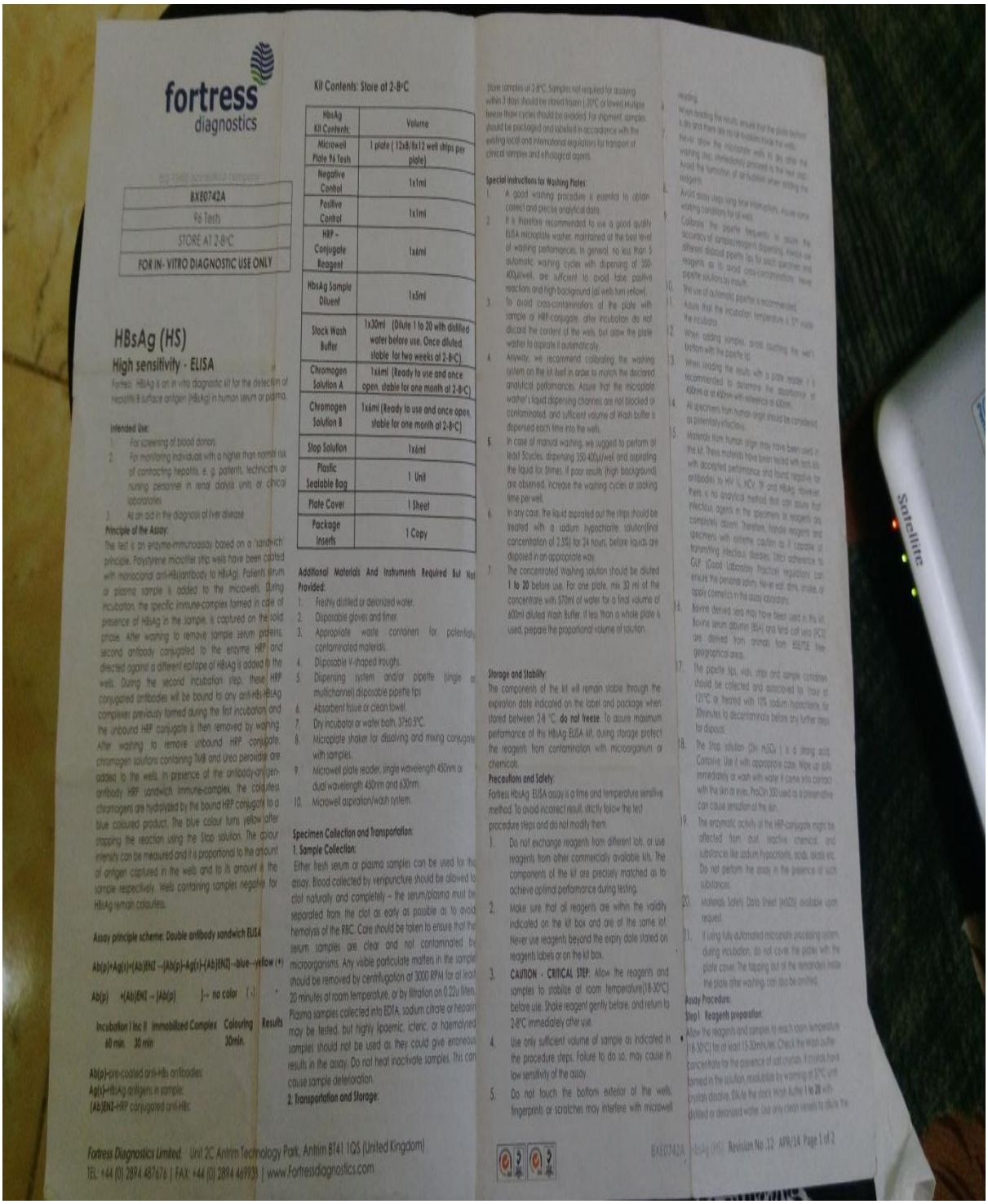


Colour plate (3)ELISA Plate

Appendix 6

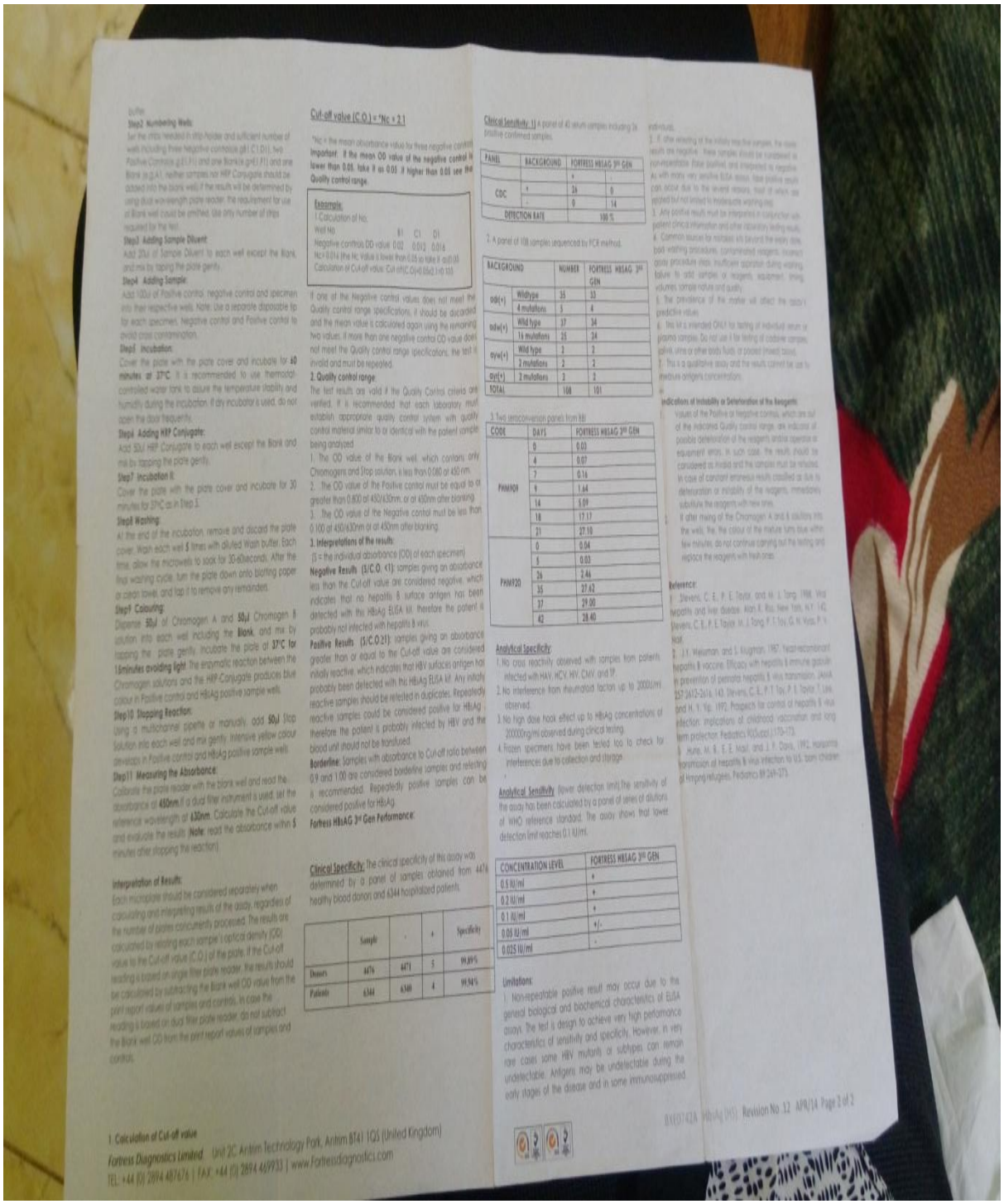
Colour plate (4)ELISA Plate

Appendix 7



Colour plate (5)Fortress diagnostics for HBsAg (HS) High sensitivity – ELISA

Appendix 8



Colour plate (6)Fortress diagnostics for HBsAg (HS) High sensitivity –ELISA

بسم الله الرحمن الرحيم

جامعه السودان للعلوم والتكنولوجيا

كليه الدراسات العليا

موافقه مستنيره

اقرار بالموافقه.....

الاسم. .....**العمر** .....

العنوان..... .

اوافق بمحض ارادتي بالمشاركه في البحث العلمي المتعلق بدراسه تردد فيروس الكبد الوبائي(ب) لدى المرضى المصابين بسرطان خلايا الكبد في ولايه الخرطوم ، وذلك باعطاء طالبه جامعه السودان اسراء محمد الحاج عبدالله 5 مل من دمي بعد ان شرح لي بانه لا يترتب عليه اي اذى جسدي او نفسي وان المشاركه في هذا البحث لن يؤثر باي حال من الاحوال علي حالتي الراهنه كما انه يحق لي بدون ابداء اسباب الانسحاب من هذا البحث في اي مرحله من مراحله

البحث باشراف:

د/ وفاء ابراهيم الحاج

اعداد الطالبه:

اسراء محمد الحاج عبدالله

.....

التوقيع.....

التاريخ.....