Sudan University for Sciences and Technology

College of Graduate Studies

Molecular Detection of Hepatitis B Virus Genotypes Among Human Immunodeficiency Virus Patients in Khartoum State Sudan

الكشف الجزيئي عن الأنماط الجينية لفيروس الالتهاب الكبدي الوبائي (ب)

بين مرضى فيروس نقص المناعة المكتسبة في ولاية الخرطوم

A dissertation submitted in partial fulfillment of M.Sc. Degree in Medical Laboratory Sciences (Microbiology)

Submitted By:
Hoda Fathi Mustafa Ali
B.Sc. of Medical Laboratory Sciences
And Technology University
2015

Supervisor:
Prof. Dr: Omer Mohamed Khalil
(Medical Microbiology)

2018

الآية

قال تعالي :-

(قَالُوا سُبْحَانَكَ لاعَلَّمْ لَنَا إلاَّ مَاعَلَّمْتنا إنكَّ أَنْتَ الْعَلِيمُ الْحَكِيم)

صدق الله العظيم

سورة البقرة الآية (32)

Dedication

To whose breastfed me the love and compassion to the symbol of love and healing to pure whiteness heart.

(My mother)

To whose spend his life working to give me a drop of love and happiness to that who pave my way to science To the Big heart I pray to God to make them one of the people of Paradise and make them from the people of bless.

(My father&uncle souls)

To the pure hearts and innocent souls to my life basil's.

(My brothers and sister)

Best wishes…

Acknowledgment

Piously my gratitude and prayers to ALMIGHTY ALLAH for the mercy that followed me during the long way of this research. I owe so much to my supervisor Dr. Omer Khalil for his immense effort not only to accomplish this work but also to inculcate the researcher's soul on me. Special thanks to my colleges' efforts and boundless support. I am thankful for Dr.Osama Mohammed boundless support and helpful advices. Special thanks to research lab accomplishment of this thesis. I am thankful for all the patients who agreed to participate in this research and my prayers to ALMIGHTY ALLAH to cure them and to bless them with long healthy life. Eventually I would like to thank anyone who contributed by any means to this research from commence, during the collection, processing of specimens or the final touches.

ABSTRACT

Hepatitis B virus (HBV) and Human immunodeficiency virus (HIV) share common routes of blood-borne transmission. There are Ten genotypes from (A-I) of HBV genome, have been identified, HBV genotypes have different geographical distribution and regarding to disease progression and response of antiviral treatment and prognosis. The objective of this study was molecular detection of HBV genotypes among HIV patients in Khartoum state. A total of 92 subjects (n=92) were included in this observational descriptive cross sectional study. The subjects were confirmed as HIV-positive by an Enzyme Linked Immunosorbent assays ELISA assay, ages ranged from 30 to 40 years, from both sexes. Serum samples were collected from the participants, and tested for HBsAg by a capture ELISA assay and for HBV DNA and genotypes by Nested Multiplexes PCR out of the 92 participants who took part in the study, 45 (48.9%) were positive for HBsAg and HBV DNA, the common genotypes 26 (57.7%) were genotype E and 19 (42.2%) were genotype D. From 45 positive patients 12 (26.6%) were coinfection genotype E and D. From the above findings we concluded that, there is a high percentage of HBV/HIV coinfection in the Sudan.

المستخلص

يشترك فيروس الالتهاب الكبدي الوبائي ب (HBV) وفيروس نقص المناعة البشرية (HIV) في طرق نقل العدوى المنقولة بالدم. هناك عشر تراكيب وراثية من (A-I) من جينوم (HBV) ، وقد تم تحديدها وراثيا.فيروس الالتهاب الكبدي الوبائي (ب) (HBV) له توزيع جغرافي مختلف فيما يتعلق بتطور المرض والاستجابة للعلاج من خلال المضادات الفيروسية والتشخيص. كان الهدف من هذه الدراسة هو الكشف الجزيئي عن الأنماط الجينية لفيروس الالتهاب الكبدي الوبائي (ب) (HBV) بين مرضى فيروس نقص المناعة المكتسبة (HIV) في ولاية الخرطوم ، الدراسة تتكون من مجموعة 92 شخصا (ن = 92) . وقد تم التأكد من الإصابة بفيروس نقص المناعة البشرية في هذه الدراسة عن طريق فحص (ELISA) ، تراوحت أعمارهم بين 30 إلى 40 سنة ، من كلا الجنسين. تم جمع عينات من المصل من المشاركين، واختبارها لـــ (HBsAg) من خلال فحص (ELISA) و (HBV DNA) من خلال Nested Multiplexes PCR. من بين 92 مشاركًا شاركوا في الدراسة ، كان 45 (48.9٪) إيجابيًا لـ (HBsAg) و (DNA HBV) ، وكان النمطان الوراثيان هما: 26 (57.7٪) النمط الجيني Eو 19 (42.2٪) هما النمط الجيني D ، بينما 12 (26.6) من النمطين (E) و (D) في نفس الوقت عدوى مشتركة. من النتائج السابقة توصلنا إلى أن هناك نسبة عالية من الإصابة بالفيروس الالتهاب الكبدي الوبائي (ب) (HBV) بين الأفراد المصابين بفيروس نقص المناعة المكتسبة (HIV) في السودان. أيضا ، كانت نسبة النمط الجيني (E) إلى غير (E) أعلى في الأفراد المصابين بالفيروس (HIV) و(HBV) عدوى مشتركة.

Table OF CONTENTS

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER FOUR

REUSLTS

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

List of Tables

List of Figures

List of Abbreviations

HBV	Hepatitis B Virus
HIV	Human immune deficiency virus
IFN-ɣ	Interferon gamma
TH cells	T helper cells
CD 8	Cluster of differentiation
WHO	World health organization
IV	Intravenous
NK	Natural killer cell
CTLs	Cytotoxic T cells
HBsAg	Hepatitis B surface antigen
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B envelope antigen
AIDS	Acquired immune deficiency syndrome
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
CCC	Covalently closed circular
ER	Endoplasmic reticulum
Bp	Base pair
Peg- IFN-α	Pegylated interferon Alfa
anti-HBs	Anti-hepatitis B surface antigen
anti–HBC	Anti-hepatitis B core antigen
IgG	Immunoglobulin G
IgM	Immunoglobulin M

anti-HBeAg	Anti-hepatitis B envelope antigen
CHB	Chronic Hepatitis B
HCC	Hepatocellular carcinoma
PCR	Polymerase chain reaction
ELISA	Enzyme linked immune sorbent assay
TNF	Tumor necrosis factor
LMV	Lamivudine
LDT	Telbivudine
ETV	Entecavir
ADV	Adefovir
TDF	Tenofovir

CHPTER ONE

INTRODUCTION AND OBJECTIVES

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1: Introduction

HBV is small circular partly double strand DNA virus, with highly compact genome. Its prototype member of hepadnaviridae family (lieven *et al*, 2000).HBV cause acute infection with mild asymptomatic and subclinical illness for about two-third of patients and one-third of adult's acute infection develop clinical symptoms and signs of hepatitis, and rarely acute liver failure in 1% of patients with acute hepatitis B and jaundice. The disease can resolve or persist to chronic Hepatitis B and cirrhosis (T jake, 2010).

There are eight genotypes from (A-H) of HBV genome, in addition to two new genotypes, I and J have been identified, HBV genotypes have different geographical distribution and regarding to disease progression and response of antiviral treatment and prognosis (Mustafa, 2014).

HBV is a viral infection cause most of the chronic liver disease globally, transmitted by sexual, vertical, and parental route. For diagnosis of HBV infection has been used serological markers consist of HBsAg, anti HBsAg, HBeAg, anti HBeAg, and anti HBcIgM and IgG, which help to know clinical phase of infection and to monitor antiviral therapy (Jeong, 2016).

HBV among immunosuppression patients infected by HIV represent public health problem regarding to the World Health Organization (WHO-2015), there was high death rate result of highly contagious capacity. 10% of HIV infected patients co-infected with HBV (Bivigou *et al*, 2018). HBV and HIV co-infection increase progression and end-stage liver disease than mono infected individuals and

sometimes the presence of HBV DNA in the liver and serum of chronically infected patients with absence of HBsAg in their blood which known as occult hepatitis B infection (OBI) (yassin *et al*, 2016).

1.2 Rationale

HBV is considered as one of the most common viruses, which can transmit through blood, blood products transfusion and organ transplantation and sexual contact. The infection may lead to serious disease and complications like liver cirrhosis, hepatocellular carcinoma and high rate of mortality and morbidity particularly among immunosuppression patients infected by (HIV) which represent public health problem regarding to the (WHO-2015). According to intergroup diver sequence HBV was classified into eight genotypes group (A-H). Distribution of HBV genotypes may differ with population migration and the time, there is incomplete information or based on small number of patients studied, although severity and progression of end stage of liver disease is more in HIV and HBV co-infection than mono infection individuals. Results of several studies HBV genotypes have different geographical distribution and regarding to disease progression and response of antiviral treatment and prognosis. Studies conducted to determine the prevalence of HBV genotypes among HIV patients.

Just one previous study is available about the genotype of HBV among HIV patients in Khartoum State obtained by Mukhalid, *et al* in Sudan, where the rate of genotypes distribution of HBV was 46.0% D, 21.6% E, 18.9% A, and 13.5% D/E recombinant (Mukhalid, *et al,* 2014). We carried this study to determine the common genotype of HBV among HIV positive individuals in Khartoum State, Sudan.

1.3 Objectives

1.3.1 General Objective

Molecular Detection of HBV Genotypes among HIV Patients in Khartoum state.

1.3.2 Specific objectives:

1.3.2.1 To detect of HBsAg in HIV patients.

1.3.2.2 To determine the common HBV genotypes among HIV using Nested multiplex PCR.

CHAPTER TWO

LITERATURE REVIEW

2. Literature review

2.1 Definition

HBV is viral infection represent one of the most common blood born pathogen word wide. HBV can progress in some patients to inactive carrier state; give rise to cirrhosis and cancer of liver leading to death (chiah, *etal*, 2018). HBV infection is considered as the major public health problem globally, 30% of the world's population gives serological evidence of current or past infection (Christian, *etal*, 2014). The virus transmitted from patients having acute or chronic infection, HBV is found mainly in the blood, vaginal secretions, semen and serous fluids of an infected individual (CDC, 2012). Transmission happens through the intravenous IV, intramuscular IM, subcutaneous SC or intradermal and premucosal exposure to infective body fluids (Heymann, 2008). HBV among immunosuppression patients infected by HIV represent public health problem regarding to the (WHO-2015), there high death rate result of highly contagious capacity. 10% of HIV infected patients co-infected with HBV. the risk of liver related disease and HBV DNA are expanding among patients with low CD4 cell counts (less than 200 cell/ml) and HIV fever the development of HBV infection (Bivigous, *etal*, ,2018) (HIV-1) and (HBV), both of them cause chronic disease, cancer and death, can be eradicated with the use of current therapies. Antiviral drug resistance often develops after patients have received treatment for some time and is usually followed by the loss of clinical benefit. Coinfection with the two viruses aggravated the negative effects (Athena, *et al*, .2015).

2.2 History

The history of modern research on viral hepatitis initiated in the year 1963(Wolfram, 2013). First discovery reported by Blumberg et al in 1967 on the novel antibody found in the serum of a multiply transfused hemophilia patient. This antibody reacted with only 1 of 24 sera against which it was tested. The reacting serum came from an Australian Aborigine hence the antigen was named the "Australia Antigen" (Catherine, *et al*, 2014).

2. 3 Classification, Virus structure and genome organization

2.3.1 Classification

HBV is small circular double strand DNA virus; it's a member of the Hebadnavirus family related to hepatotropic DNA virus's family which share many genetic and biological features (Frances, *etal*, 2004). One strand is complete circular with over lapping genes called ((minus)) encoded for (pre-s, surface, core) structural proteins and (polymerase x) replicative proteins, the other strand is short and variable in length called ((plus)) (J.Y.N.2003). According to intergroup diver sequence HBV has been classified into eight genotypes group (A-H) (Scott, *etal*, 2007).In addition to new classification of HBV genotypes appears 10 genotypes (from A to J). A redefinition of sub-genotypes was recently suggested with the creation of Quasi-sub-genotypes (QS) QS-A3, QS- B3, QS-C2, regrouping clusters in each genotype, and a recombine sub-genotype within the genotype (Berthold, *et al*, 2018).

2.3.2: Genome

Containing a partially double stranded circular DNA genome, it is a 42-nm enveloped virion (known as Dane particle), with an icosahedral nucleocapsid core, its genome is the smallest of all known animal DNA

viruses. The envelope contains a protein called the surface antigen (HBsAg), which is important for laboratory diagnosis and immunization, within the core is a DNA-dependent DNA polymerase. The genome contains four genes (four open reading frames) that encode, surface envelope protein, core (nucleocapsid) protein, DNA polymerase, and X protein, an activator of viral RNA transcription. DNA polymerase has both RNA-dependent (reverse transcriptase) and DNA-dependent activity (Stefan and Francis, 2005).

Patient's serum on the Electron microscopy appears three different types of particles, a few 42-nm virions and many 22-nm spheres and long filaments 22 nm wide, which are composed of surface antigen, HBV is the only human virus that produces these spheres and filaments in such large numbers in the patient's blood in addition to HBsAg, there are two other important antigens, the core antigen (HBcAg) and the envelope antigen (HBeAg), both of which are located in the core (nucleocapsid) proteins but are antigenically different (Stefan and Francis, 2005).

2.4 HBV nucleocapsid

HBV consist of icosahedral capsid replicating via reverse transcription. It has a diameter of 36 nm and is formed by one protein species (C protein). Assembly of the capsid happen in the cytosol and results in packaging of a 3.5 kb RNA molecule together with viral and cellular factors. Then newly capsid cannot be enveloped to induced transcription is required to synthesis of the viral DNA genome in the lumen of the capsid (Vanlandschoot, *etal*, 2003). The HBV nucleocapsid or core antigen is highly immunogenic during infection and after immunization (Bruss, 2004).

2.5 ENVELOPE

Enveloped proteins of the virus coded by surface proteins, in addition to two other related proteins (large S and middle S). (Uede, *et al,* 1991). Outer envelope coded by S gene have important role, which induction of protective neutralizing antibody response and any mutation in the S gene effect directly in the production of C gene products which considered as target for immune T cell involved in virus clearance (Howord,1995).

2.6 Viral replication

HBV replicates by reverse transcription are similar to retro virus in basic cycle and genome organization (Micheal and Heinz, 1993). HBV has unique replication strategy. start with the virion binds to a specific receptors at the surface of hepatocyte number of candidate receptors has been identified, including the transferrin receptor, the glycoprotein receptor molecule , and human liver endonexin, the mechanism of HBsAg binding to a specific receptor to enter cells has not been established yet (Micheal and Heinz, 1993).

Viral nucleocapsids enter the cell and reach the nucleus, where the viral genome is transferred, in the nucleus, second strand DNA synthesis is completed and the gaps in both strands are repaired to yield a covalently closed circular (ccc) super coiled DNA molecule serve as a template for transcription of viral RNAs (Micheal and Heinz, 1993).

These transcripts are polyadenylated and transported to the cytoplasm, where they are translated into the viral nucleocapsid and pre-core antigen (C, pre-C), polymerase (P), envelope L (large), M (medium), S (small) and transcriptional transactivating proteins (X) (Micheal and Heinz, 1993).

The envelope proteins insert themselves as integral membrane proteins into the lipid membrane of the endoplasmic reticulum (ER), the 3.5 kb species, spanning the entire genome and termed pre-genomic RNA (pgRNA), is packaged together with HBV polymerase and a protein kinase into core particles where it serves as a template `of reverse transcription of negative strand DNA (Ganem and Schneider, 2001).

The new, mature viral nucleocapsids can then follow two different intracellular pathways, one of which leads to the formation and secretion of new virions, while the other leads to amplification of the viral genome inside the cell nucleus (Ganem and Schneider, 2001).

In the virion assembly pathway, the nucleocapsids reach the ER, where they associate with envelope proteins and bud into the lumen of the ER, from which they are secreted via the Golgi apparatus out of the cell (Ganem and Schneider, 2001).

In the genome amplification pathway, the nucleocapsids deliver their genome to amplify the closed circular DNA (cccDNA). The X protein contributes the efficiency of HBV replication by interacting with different transcription factors, and is capable of stimulating both cell proliferation and cell death (Ganem and Schneider, 2001).

The HBV polymerase is a multifunctional enzyme, which synthesis DNA by using either RNA or DNA templates, the product of the P gene are involved in multiple functions of the viral life cycle, including a priming activity to initiate minus strand DNA synthesis, a polymerase activity, a nuclease activity which degrades the RNA strand of RNA-DNA hybrids and the packaging of the RNA pre-genome into nuleocapsids (Ganem and Schneider, 2001).

2.7 Antigenic structure

HBV possess three coat proteins all of them are contain HBsAg, which is highly immunogenic and stimulate anti-HBs (humoral immunity). The viral proteins structure triggersspecific T-lymphocytes able to eliminating HBV infected cells.HBsAg is heterogeneous antigenically, with a common antigen designated, and two pairs of mutually exclusive antigens, d, y, w (including several sub determinants) and r, resulting in 4 major subtypes, adw, ayw, adr, ayr (Hollinger *and Liang*, 2001).

The c antigen (HBcAg) is present on the surface of core particles, HBcAg and core particles are not present in the blood in a free form, but are found only as internal components of virus particles. The core antigen shares it sequences with the e antigen (HBeAg), identified as soluble antigen but no cross reactivity between the two proteins are observed (Robinson, 1999).

2.8 Epidemiology

Hepatitis B is considered as one of the most common infectious diseases worldwide. There are 350 million chronic HBV carriers globally have been estimated. The prevalence of chronic HBV infection differs geographically, from high (>8%), intermediate (2-7%) to low (<2%) prevalence (Jinlin, *et al*, 2005).

High prevalence rate of chronic HBV infection effect more than 8% of population is common in Asia pacific and Sub-Saharan African regions populations, 45% of the world's population live in an area of high prevalence. Inter mediate prevalence rate which effect (2%-7%) of

populations include North Africa and Middle East, parts of eastern and south Asia. Low prevalence populations include Australia, Asia, Northern and Western Europe, Japan, North America and some countries of South America make up minority of global population (~12%) (Jennifer, *etal*, 2015).

The eight genotypes from (A-H) of HBV genome, in addition to two new genotypes, I and J have been identified, HBV genotypes have different geographical distribution and regarding to disease progression and response of antiviral treatment and prognosis (Mustafa,2014).

Genotype A wide spread in northern Europe and sub-Saharan and western Africa, while genotype B and C distributed in Asia, genotype D is predominant in Africa, Europe and Mediterranean countries and genotype G spread in France, The united states, Germany, and genotype H is reported in central and south America, genotype I reported in the Ryukyu island in Japan (Mustafa, 2014).

2.8.1 Prevalence of HBV among HIV

Since 2015 about 1% of HBV individuals (2.7 million people) are infected with HIV the global prevalence of HBV among HIV infected person is 7.4% (WHO, 2018).

Chronic HBV among HIV individuals increase morbidity and mortality rate. HIV positive patients are common in co-infected with HBV due to shared routs of transmission (Henery, *et al*, 2014).

Identification of HBV genotypes are important in association exist between clinical outcomes and efficacy of treatment (Mustafa, 2014).

HBV genotypes in a study applied in 2002 on HIV infected patients with HBV chronic infection shown a total of 28 of 1100 individuals (prevalence 2.5%) were found to be HBsAg and HBV-DNA positive. HBV genotypes could be determined

in 23 of them. HBV-A was the most common (57%), followed by HBV-D (39%). HBV-A predominated among homosexual men (67%) while HBV-D predominated among intravenous drug users (67%) (Myate, *et al,* 2004).

2.9 Mode of transmission

The most commonly transmitted of Hepatitis B virus by (perinatal transmission) from mother to the baby, or exposure to infected blood, HBV can survive outside the body for at least 7 days and the incubation period vary from 30 to 180 days (Who.2018). Transmission of HBV can be vertical through childbirth or horizontal transmission by inter venous drug abuse or by sexual contact or occupational exposure .The main three routs of transmission in health care: 1- needle skin injury by infected blood or body fluid, 2- direct contact by non-intact skin or mucous membrane or body fluid, 3- by indirect by contaminated non intact skin or surface (Bineeta, *et al*, 2018).

2.10Viral pathogenesis and immunity

HBV is a microinflammatory liver disease with different severity, infection associated with chronic liver disease that lead cirrhosis and hepatocellular carcinoma is often refer to persistent infection of HBV (Francis, *et al,* 2010). Hepatitis B infection has different clinical manifestations depending on the patient's age at infection and immune status, and the stage at which the disease is recognized. As the Virus considered non cytopathic virus the damage that occur appear to be mediated by the immune system attack of infected hepatocyte in order to clear the infection, during the incubation phase of the disease 6 to 24 weeks, patients may feel unwell with possible nausea, vomiting, diarrhea, anorexia and headache. Patients may then become jaundiced although low grade fever and loss of appetite may improve, Sometimes HBV infection

produces neither jaundice nor obvious symptoms (Hollinger and Liang, 2001).

The asymptomatic cases can be identified by detecting biochemical or virus specific serologic alterations in their blood. They may become silent carries of the virus and constitute a reservoir for further transmission to others. Most adult patients recover completely from their HBV infection, but about 5 to 10% will not clear the virus and will progress to become asymptomatic carriers or develop chronic HBV possibly resulting in cirrhosis and/or liver cancer and rarely, others may develop fulminant hepatitis and die (Robinson, 1999).

People who develop chronic HBV may develop significant and potentially fatal disease. In general, the frequency of clinical disease increases with age, whereas the percentage of carriers decreases, persistent or chronic HBV infection is among the most common persistent viral infections in humans. More than 350 million people in the world today are estimated to be persistently infected with HBV, a large fraction of these are in eastern Asia and Sub-Saharan Africa, where the associated complications of chronic liver disease and liver cancer are the most important health problems. A small number of long established chronic carriers apparently terminate their active infection and become HBsAg negative, Survivors of fulminant hepatitis rarely become infected persistently, and HBsAg carriers frequently have no history of recognized acute hepatitis (Robinson, 1999).

Chronic hepatitis appears result of cellular immune response that destroys infected hepatocytes and doesn't recognize the virus from remaining infected hepatocyte (Xiaodong, *et al*, 2006).

The adaptive immune response mediates the liver disease associated with viral hepatitis, antigen-nonspecific inflammatory cells trigger cytotoxic T cell, which induce immune pathology, and platelets enhance the accumulation of CTLs in liver, recurrent immune-mediated liver damage after long periods lead to development of cirrhosis and hepatocellular carcinoma due to low level cycle destruction result of inefficient T cell response incapable to complete clearance of HBV from the liver (Guidotti and Chisari, 2006).

Innate immunity trigger through recognition of early phase of viral infection, activation of different type of families of cellular receptor (toll-like receptors [TLRs],RIG-1) cause rapid of antiviral cytokines, such as (IFN)–α and natural killer (NK) cell which limits the initial spread of HBV. The efficient recruitment of adaptive immune system need the activation of innate immunity, adaptive immunity have critical role in clearance of the HBV infection through production of CD4 which secret cytokines required for efficient development of effectors CD8, CTLs and B-cell antibody production (Anthony, *et al*, 2015).

2.10.1 Acute hepatitis B infection

The acute form of the disease often resolves spontaneously after a 4-8 weeks illness. Most patients recover without significant consequences and without recurrence. However, a favorable prognosis is not certain, especially in the elderly who can develop fulminating, fatal cases of acute

hepatic necrosis. Young children rarely develop acute clinical disease, but many of those infected before the age of seven will become chronic carriers, the incubation period varies usually between 45 and 120 days, with an average of 60 to 90 days. The variation is related to the amount of virus in the inoculums, the mode of transmission and host factors (Chisari and Ferari, 1997).

The hallmark of acute viral hepatitis is the striking elevation in serum transaminase (aminotransferase) activity. The increase in aminotransferase, especially ALT, during acute hepatitis B varies from mild to moderate increase of 3 to 10 fold to striking increase of more than 100 fold (Chisari and Ferari, 1997).

In patients with clinical illness, the onset is usually insidious with vague abdominal discomfort, nausea and vomiting, sometimes arthralgia and rash, often progressing to jaundice, fever may be absent or mild (Chisari and Ferari, 1997).

The citric phase of acute viral hepatitis begins usually within 10 days of the initial symptoms with the appearance of the dark urine followed by a pale stools and yellowish discoloration of the mucus membrane, conjunctiva, sclera, and skin. Jaundice became apparent clinically when the total bilirubin level exceeds 2 to 4 mg/dl. It is combined by hepatomegaly and splenomegaly. About 4-12 weeks thereafter, the jaundice disappears and the illness resolves with the development of natural, protective antibodies (anti-HBs), in about 95% of adults (Hollinger and Liang, 2001).

Acute HBV is characterized by presence of anti–HBC IgM serum antibodies converting to IgG with convalescence and recovery, and the transient presence of HBsAg, HBeAg, and viral DNA, with clearance of these markers followed by seroconversion to anti-HBsAg and anti-HBeAg. More than 90% of adult onset infection cases fall in to this category. The remaining 5 to 10 % of adult onset infection and over 90% of cases of neonatal infection become chronic and may continue for the life span of the patient (Mahony, 1999).

2.10.2 Chronic hepatitis B infection

Chronic HBV infection is defined as persistence of hepatitis B surface antigen (HBsAg) for more than six months, high levels of HBV DNA, and presence of (HBeAg) in the serum. Chronic HBV infection occurs in approximately five to ten percent of individuals with acute HBV infection. Long-term effects of chronic HBV infection include cirrhosis, liver failure, and HCC. HBV infections acquired by infants or children are significantly more likely to progress to chronic HBV infections as compared to adults (Keeffe *et al,* 2008).

Chronic HBV can cause serious destruction to the liver and it contributes greatly to the world wide burden of the disease states. Surprisingly, some of the patients infected persistently may have no clinical or biochemical evidence of liver disease, while other may show signs of easy fatigability, anxiety, anorexia, and malaise (Mahony, 1999).

2.10.3 Hepatocellular carcinoma

A number of HBV patients with chronic hepatitis will develop HCC. Persons at increased risk of developing HCC include adult male and chronic HBV with cirrhosis that contracted hepatitis B in early childhood. Only about 5% of patients with cirrhosis develop HCC. On the other hand between 60 and 90% of HCC patients have underlying cirrhosis (Hollinger and Liang, 2001).

The incidence of HCC varies with geography, race, age, and six. HCC is responsible for 90% of the primary malignant tumor of the liver observed in adult. Globally, it is the seventh most frequent cancer in males and ninth most common in females. Liver cancer is a cause more than 500,000 deaths and annually throughout the world, with a male: female ratio 4:1. The frequency of HCC follows the same general geographic distribution pattern as that of persistence of HBV infection. The age of distribution of patient with clinically recognized tumors suggests that these tumors appear after duration of about 35 years of HBV infection (Robinson, 1995).

Patient who develop HCC as a result of malignant transformation of hepatocyte have a mean 5 year survival rate of 25 to 60%. This variation depends on the size of the tumor and the presence or absence of alpha-fetoprotein (Hollinger and Liang, 2001).

2.10.4 Role of HBV genotype in pathogenesis

Possible pathogenic differences among hepatitis B virus (HBV) genotypes have been observed (Jia, *et al*. 2000).

Eight HBV genotypes (HBV genotypes A–H) have been identified by a sequence divergence greater than 8% in the entire HBV genome in addition of genotype I and J are recently discovered, The geographic and ethnic distributions of HBV genotypes and subtypes determining disease progression. Genotype A HBV is found in North America, Southern and East Africa, and Western Europe HBV subgenotype (A1 or Aa) of HBV genotype A is associated with HCC in young men who are usually HBeAg-negative and anti-HBe-positive, have low levels of HBV DNA and rarely have cirrhosis while HBV subgenotype A2 or Ae (European HBV subgenotype), which is associated with HCC in older European individuals. , HBV genotype B, is divided into two major groups: Bj, which is found in Japan, and Ba, which is found in the rest of Asia, subgenotype Ba is associated with older age at the time of HBeAg seroconversion, a higher risk for HCC, more severe liver disease and a higher frequency of basal core promoter (BCP) mutations as compared with HBV subgenotype Bj. HBV genotype C is found in Asia, according to compelling data from multiple population-based and clinic-based prospective trials that show that HBV genotype C is independently associated with a higher risk of HCC than HBV genotypes A2, Ba and Bj or D. HBV genotype D, which is distributed in Northern Africa, Southern and Eastern Europe, and the Middle East, is associated with early HBeAg seroconversion, persons infected with HBV genotype D in the inactive hepatitis B phase are likely to remain in this phase without developing complications of liver disease or HCC. In one study, 97% of those patients with minimal or no fibrosis or inflammation on liver biopsy had no histological progression upon repeat liver biopsy after 4 years of follow-

up. Infections with HBV genotypes E, G and H are uncommon (Beom, *et al*. 2011).

2.11 Laboratory diagnosis

HBV diagnosis is carried out by testing for a series of serological markers of HBV and by additional testing to exclude alternative etiological agents such as HBV A and C viruses. Serological tests are used to differentiate acute, self-limited infections from chronic HBV infections and to monitor vaccine-induced immunity. Nucleic acid testing for HBV-DNA used to quantify HBV viral load and measure the effectiveness of therapeutic agents. The envelope proteins are surface glycoproteins called hepatitis B surface antigen (HBsAg). In virus-infected liver cells, HBsAg is produced in excess and secreted into the blood; refer as a marker for active infection and infectivity. Currently, recombinant HBsAg is used for HBV vaccination, and the development of antibody to HBsAg is typically associated with protective immunity. The core open reading frame encodes a polypeptide that is expressed as either the hepatitis B e antigen (HBeAg) or the viral capsid protein (HBcAg). The presence of detectable HBeAg in serum or plasma is associated with high levels of HBV replication, greater infectivity and an increased risk of hepatic fibrosis (Mel, *et al*. 2005).

HBsAg first appears in the blood during the incubation period, while the virus is actively 'replicating' in liver cell. The HBsAg disappears within 3 months, the HBeAg and HBV DNA can detect in blood while the virus actively replicating in the liver in acute infection. IgM antibody specific to HBcAg (ani-HBc), rise first refer to primary indicator of acute infection remains for at least 6 months the IgG component of anti-HBc persists for

life. Anti-Be is the second antibody appear associated with rapid clearance of HBeAg and decline. Anti-HBs become not detectable for 3-6 months after acute infection associated with resolution of the illness (David and Stephan. 1998).

Table 2.1

Primary markers for the diagnosis of acute hepatitis B infection

Diagnosis	HBV Antigens		HBV DNA	Anti-HBV Antibodies			
	HBsAg	HBeAg		Anti-HBc		Anti-HBe	Anti-HBs
				IgM	Total Ig		
Acute HBV	++	+	+	++	+	-	-
Resolving							
early	-	±	+	±	±	±	±
late	-	-	-	-	+	+	+
Immune							
vaccination	-	-	-	-	-	-	+
Past infection	-	-	-	-	+	-	+

Table 2.2

Primary markers for the diagnosis of chronic hepatitis B infection

Diagnosis	*HBV Antigens*		*HBV DNA*	*Anti-HBV Antibodies*			
	HBsAg	*HBeAg*		*Anti-HBc*		*Anti-HBe*	*Anti-HBs*
				IgM	*Total Ig*		
HBeAg positive							
chronic hepatitis B	+	+	++		++	-	-
HBeAg negative chronic hepatitis B	+	-	+/++		++	±	-

Occult HBV infection is defined as the persistence of HBV DNA in the liver with detectable (200 IU/ml) or undetectable HBV DNA in serum of individuals who are HBsAg-negative, which defined by the indefinite persistence of antibodies to HBV core antigen (anti-HBc) ; thus anti-HBc is considered as overall marker of exposure to HBV infection. In absence of HBsAg serum Anti-HBsAg is indicate protective immunity against HBV acquired by vaccination (anti-HBc-negative) or natural infection (anti-HBc-positive) (Ferruccio *et al*, 2010).

The current diagnostic repertoire of HBV serum markers includes viral antigen and antibodies detected or measured by immuneassay and viral DNA measured by Polymerase chain reaction (PCR) based amplification assays. In addition, we might detect both viral antigens (HBsAg, HBcAg) and viral nucleic acid in liver tissue by immunohistochemistry and PCR. When selecting the most appropriate assay it is important to note that their relevance is related to the biological properties of the particular marker they detect (Ferruccio *et al*, 2010).

2.12 Treatment

Treatment of HBV infection based on the different strategies of vaccination, treatment and prevention of the transmission in order to eradicate the infection (Gutam.2017). The therapeutic goal in HBV infected patients is sustained HBsAg clearance. It is preceded by the loss or significant suppression of HBV replication and leads to inhibition of the progression of liver fibrosis, normalization of biochemical indicators of liver damage, decrease the risk of HHC prolongation of survival, prevention of HBV infection in the transplanted organ in post-transplant patients, enhancement of the quality of life, inhibition or reversal of extrahepatic changes associated with HBV infection, and cessation of the spread of HBV infections (Robert, *et al*, 2017).Drugs approved in the European Union for the therapy of HBV infection consist of:

- interferon (IFN):
  - natural interferons,
  - α-2a and α-2b (IFNα-2a and IFNα-2b),
  - pegylated α-2a (PegIFNα-2a);
- analogues (NA):
  - nucleoside analogues: lamivudine (LMV), telbivudine (LdT) and entecavir (ETV),
- Nucleotide analogues: adefovir (ADV), tenofovirdisoproxil (TDF) and tenofoviralafenamide (TAF).

PegIFNα-2a therapy should continue for 48 weeks, in HBeAg-positive patients, infected with genotypes A or D – when the qHBsAg level fails to decrease after 12 weeks of treatment, infected with genotypes B or C when the qHBsAg level

exceeds 20,000 IU/ml after 12 weeks of treatment. regardless of the genotype or in cases where the genotype is unknown – when the HBV-DNA level fails to decrease by at least 2 $\log_{10}$ after 12 weeks of treatment or when the qHBsAg level is higher than20,000 IU/ml after 24 weeks of treatment and in HBeAg-negative patients regardless of the genotype when the qHBsAg level fails to decrease by any degree after 12 weeks of treatment or when the level of HBV-DNA fails to decrease by at least 2 $\log_{10}$ after 12 weeks of treatment (Robert, *et al*, 2017).

2.13 Prevention and Control

2.13.1 Vaccination against HBV infection:

Since 1981 a vaccine against HBV is available. Considered safe and effective, this vaccine has a protective efficiency of 90–95% (Mahoney, 1999). All health care workers are required to be vaccinated against HBV (Mahoney.1999). Especially surgeons and surgical trainees, nurse and laboratory technicians who are at a high risk of infection (Taylor and J.M, 2006).

The major factors to prevent and control hepatitis B are the knowledge and awareness on the part of healthcare providers, at-risk populations, the public, and policy makers. Sufficient understanding about the seriousness of this public health problem, control, and surveillance programs (Abigail, *et al*. 2010).

CHAPTER THREE

MATERIALS AND METHODS

3. Materials and Methods

3.1 Study design

Descriptive Cross sectional study.

3.2 Study area and duration

Patients attending different Total Lab Care laboratories in Khartoum state, Sudan in the period from Jan 2017 to June 2018.

3.3 Study population

Study was conducted in HIV patients.

3.4 Sample size

Ninety two (n=92) blood samples were collected in EDTA containers.

3.5 Sampling technique

HIV patients were randomly selected**.**

3.6 Inclusion criteria

HIV patients were included.

3.7 Exclusion criteria

Negative HIV patients.

3.8 Data collection

Data was collected using Hospital records

3.9 Data analysis

Collected data was analyzed by using SPSS program version 16.

3.10 Ethical consideration

1-Approval has been taken from Sudan University of Science and Technology, College of Postgraduate and Medical Microbiology Department.

2-Approval has been taken from Total lab Care laboratories.

3 -Verbal consent has been taken from patients.

3.11 Laboratory work

3.11.1 Sample collection

Five milliliters (ml) of blood was collected into EDTA containers for molecular and serological procedures and was centrifuged at 3000 rpm for 5minutes to obtain plasma which was transferred into plain containers and stored at -20 °C until processing.

3.11.2ELISA Detection of HBsAg in HIV patients

The ELISA assay was used to detect specific HBsAg. Commercial ELISA kits were purchased from CTK biotech, Inc (San Diego, CA, USA).

3.11.3. Preparation of Reagents

Test samples, controls, conjugate, diluted washing solution, and substrate were allowed to warm to room temperature (20 – 25ºC) before running the assay.

All liquid reagents were mixed before use. The concentrated washing solution, (50ml), was diluted with (450 ml) of distilled water.

3.11.4 Test Procedure

The ELISA procedure was done according to the manufacturer's instructions. In brief, 50 µl of the plasma was incubated at 37C for 60 minutes in 96 well microplate coated with rabbit monoclonal antibodies reactive to HBsAg (anti HBs). Subsequently, the wells were washed (three times) to remove residual plasma. 50 µl of anti HBs conjugated AB was added and incubated at 37C for 60 minutes. The wells were washed (three times) to eliminate unbound conjugate, 50µl of enzyme substrate and chromogen was added and incubated at 37C for 60

minutes. 50 µl diluted stop solution (sulpheric acid) was added and the plate was read at 450 nm as indicated by the manufacturer.

3.11.5 Interpretation of the Result

The Cut-off value was estimated by calculating the mean (m) absorbance value of negative control x 2.1. Cut-off = mean of negative control. X 2.1 for the validity of the accomplishment the following is required:

a- Negative control means: absorbance of individual negative control values must be less than 0.9

b- Positive control means: it must be greater than 1.1

c- Equivocal means: absorbance of individual equivocal values must be between 0.9- 1.1

3.11.6 DNA extraction

DNA extracted from plasma by using DNA extraction kits according to manufacturing instruction (Intron biotechnology Korea).

3.11.7 Nested Multiplex PCR (PCR) for detection HBV genotypes

HBV genotypes were detected by amplification of pre-S1 through S genes using universal primers, (P1) sense primer, (S1-2) antisense primer, for detection of all HBV genotypes according to described methods by Naito *et al.* (2001) the reaction.

Total volume 25 µl in the PCR reaction, containing 5 µl of DNA mixed with2µl 10 pmol of each primers forward and reverse primer (table 1), 5 µl of 2 mM dNTPmix, 2 µl of 25 mM MgCl2, 2.5 U Taq, 1x buffer and ddH2O DNA

Polymerase (intron biotechnology South Korea).The thermo cycler (heal force, China) was programmed to incubate the samples for initial denaturation at 94°C for 5 minutes, followed by 40 cycles consisted of denaturation at 94 °C for 1 minutes, annealing at 55°C for 1 minutes and elongation at 72 °C for 2 minutes. The final Elongation was 72 °C for 5 minutes. Genotyping Method Genotyping system based on nested PCR, using type specific primers for determination of six genotypes A through F of HBV according to previous method described by Naito *et al*. (2001). The nested PCR primers were designed on the basis of the conserved nature of the nucleotide sequences in regions of the pre-S1 through S genes. The genotypes can determined according to differences in the sizes of amplified DNA, in respective of the six HBV genotypes (Table 1). Two nested PCRs were performed in deferent mixture for each sample. Mix A applied for identification of genotypes A, B, C and Mix B for genotypes D, E, F. 3µL aliquot of the first-round PCR product was added to each of mix A and mix B. The nested PCR mixture made of 25 µl in the PCR reaction, containing 5 µl of cDNA mixed with 2µl 10 pmol of each primers forward and reverse primer (Table 1), 5 µl of 2 mM dNTPmix, 2 µl of 25 mM MgCl2, 2.5 U Taq, 1x buffer and ddH2O DNA Polymerase (intron biotechnology South Korea). The nested PCRs were amplified for 40 cycles with the following parameters; preheating at 94°C for 5 minutes, 20 cycles of amplification at 94°C for 30 s, 58°C for 30 s, and 72°C for 40 s, and an additional 20 cycles of 94°C for 20 s, 60°C for 30 s, and 72°C for 40 s, with the final elongation at 72°C for 5 minutes. PCR products were electrophoresed in a 1.5% agarose gel and stained with ethidium bromide. The PCR bands were then visualized by UV trans illuminator. The sizes of PCR products were estimated according to the migration pattern of a 50bp DNA ladder. The genotypes of HBV were determined according to the amplified size of PCR product (table 1).

Table (3.1) The table show revers and forward primers sequence, position and expected size band for Pre S gene first PCR round product according to previous method described by Naito *et al*. (2001)

First PCR round					
P1	universal	sense	5'-TCA CCA TAT TCT TGG GAA CAA GA-3'	(2823–2845 nt)	1063bp
S1	universal	anti-sense	5'-CGA ACC ACT GAA CAA ATG GC-3	(704-685 nt)	

Table (3.2) the table show primers sequence, position and expected size band for HBV genotypes second round according to previous method described by Naito *et al*. (2001)**.**

Second PCR round						
MIX A	BA1R	specific type A	sense	5'-CTC GCG GAG ATT GAC GAG ATG T-3'	(134-113 nt,)	68 bp
	BB1R	specific type B	sense	5'-CAG GTT GGT GAG TGA CTG GAG A-3'	(345-324 nt,)	281 bp
	BC1R	specific type C	sense	5'-GGT CCT AGG AAT CCT GAT GTT G-3	(186 - 165 nt)	122bp
	B2	specific genotype A to E	Antisense	5'-GGC TCM AGT TCM GGA ACA GT-3'	(67– 86, nt,)	
MIX	BD1	specific type D	sense	5'-GCC AAC AAG GTA GGA GCT-3'	(2979–2996	119bp

B					nt,)	
	BE1	Specific type E	sense	5'-CAC CAG AAA TCC AGA TTG GGA CCA-3'	(2955–2978 nt)	167bp
	BF1	specific type F	sense	5'-GYT ACG GTC CAG GGT TAC CA-3'	3032–3051 nt)	97 bp
	B2R	Specific types D to F	Antisense	5'-GGA GGC GGA TYT GCT GGC AA-3'	(3097-3078 nt)	

CHAPTER FOUR

RESULTS

4. Results

4.1.1. Detection of HBsAg among HIV patients:

Forty five out of 92 (48.9%) patients, 30 (66.7%) were males and 15 (33.3%) were females, showed HBsAg in their plasma samples (Table 4) and (Figure 4.2).

4.1.2 Nested Multiplex PCR for detection HBV genotypes results:

HBV DNA was detected in 45\92 (48.9%), from HIV patients, 26 (57.7%) were genotype E, 19 of them were males and 7 were females, while 19 (42.2%) were genotype D, 11 of them were males and 8 were females, 12\45 (26.6%) were genotype E and D co-infection 11 of them were males and 1 was female (Table 5), Figure (4.3).

Table (4.1) Detection of HBsAg in plasma of HIV in Khartoum state using ELISA during 2018.

Patients	**Total**	**No +ve**	**% +ve**
Males	**30**	**30**	**66.7%**
Femals	**15**	**15**	**33.3%**
Total	**45**	**45**	**100%**

Table (4.2) Common HBV genotypes among HIV patients in Khartoum State

Genotypes	No +ve	No –ve	Total tested	% +ve	Males +ve	Females +ve
E	**26**	**19**	**45**	**57.7%**	**19**	**7**
D	**19**	**26**	**45**	**42.2%**	**11**	**8**
D&E co-infection	**12**	**33**	**45**	**26.6%**	**11**	**1**

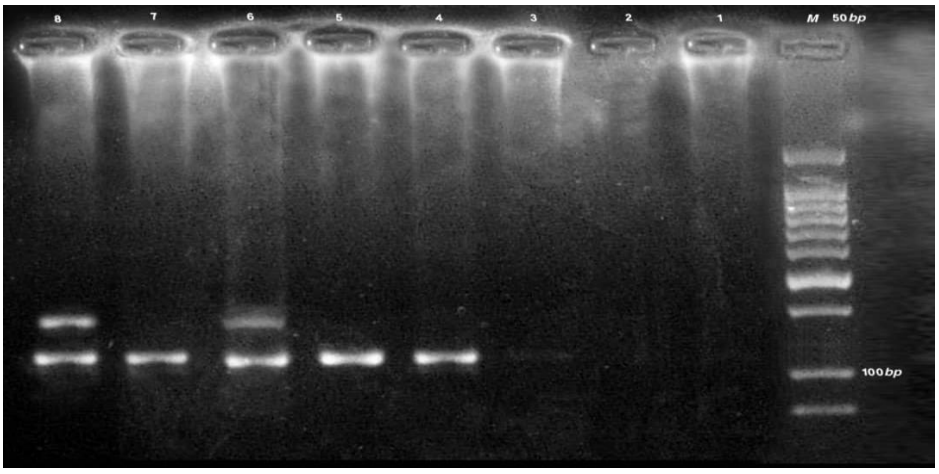


Figure (4.1): HBV genotypes among HIV patients

lane 1, 2 negative control lane 3, 4, 5 and 7

Positive for HBV genotype D (119bp) lane 6 positive control for genotype

(D119 bp and E 162bp) lane number 8 positive sample for genotype

(D 119bp and E 162 bp).

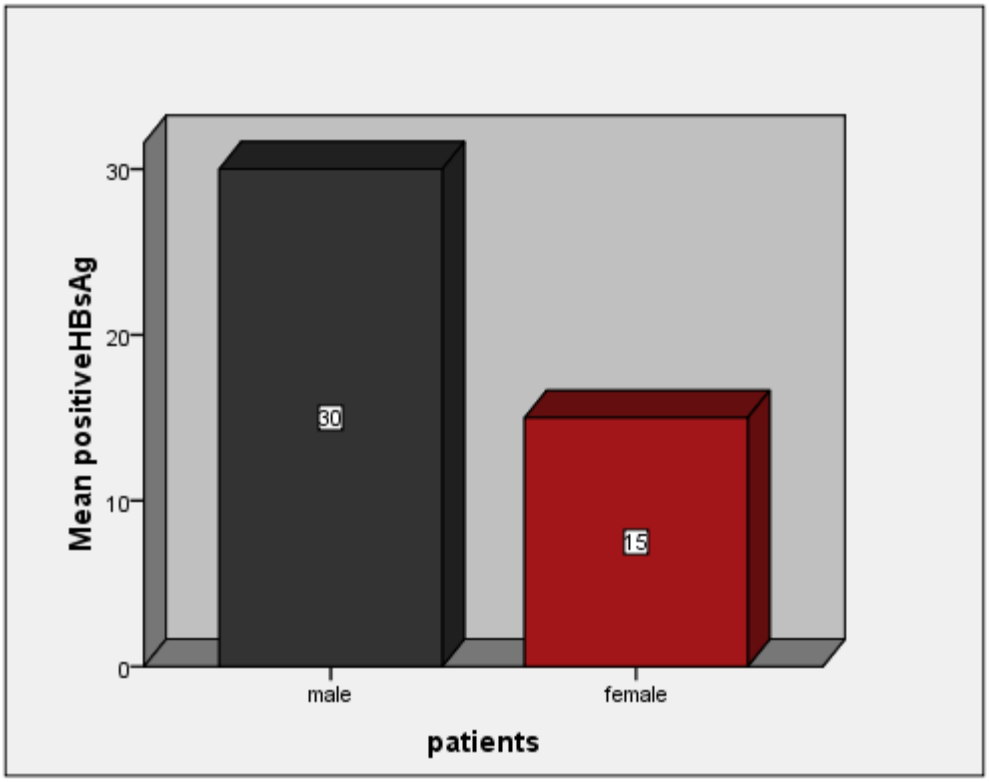


Figure (4.2) Distribution of HBsAg in plasma of HIV among Males and females in Khartoum State using ELISA

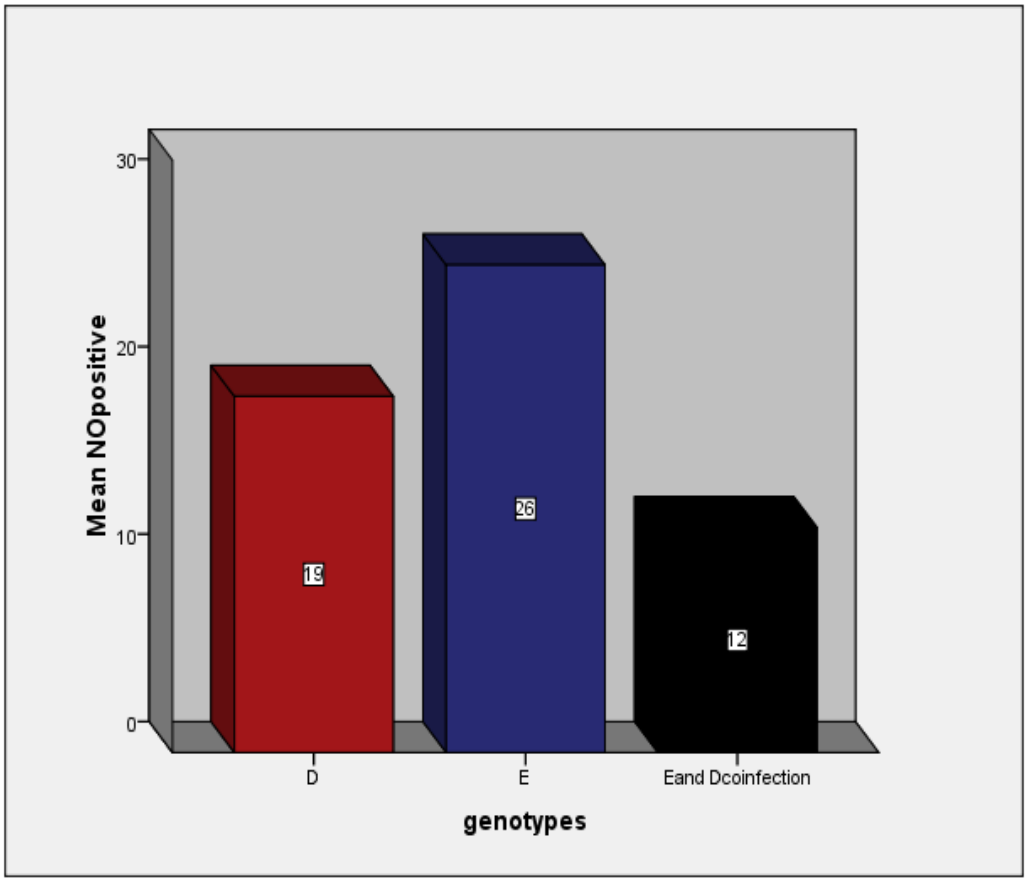


Figure (4.3) Prevalence of the common HBV genotypes among HIV in Khartoum State using nested multiplex PCR.

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

5.1 Discussion

HBV is a microinflammatory liver disease with different severity, infection associated with chronic liver disease that lead to cirrhosis and HCC, is often refer to persistent infection of HBV (Francis, *et al*,2010).

The study was conducted to determine the prevalence of HBV genotypes among HIV patients in Khartoum State. Little information is available about the genotype of HBV among HIV patients in Khartoum State.

The prevalence of serological markers of HBV exposure in HIV-infected individuals varies according to the geographical area and the risk rates of the studied population. Knowledge on regional HBV prevalence is important for providers. HIV-infected patients measured in this study (n=92) were randomly selected for the present study 63 of them were males (68.4%) and 29 were females (31.5%) with mean age of 35. HBV DNA was detected in 45 (48.9%) with an overt HBsAg-positive infection, the result is similar to the study obtained by Yassin *et al* in Sudan, Eighty-seven HIV-positive patients (n=87) were randomly selected 50 of them were males (57.5%) and 37were females (42.5%), with mean age of 35years, the result was13(14.9%) positive HBV DNA and 14(16.1%) positive HBsAg, 8% occult HBV (Yassin, *et al*. 2016). The prevalence of HBV infection among HIV-infected patients in this study in comparing with our study and other similar study showed a result of lower prevalence in females than males which both of them were in Sudan with same geographical distribution. HBV DNA was detected in 45\92 (48.9%), from HIV patients, 26 (57.7%) were genotype E, 19 of them were

males and 7 were females, while 19 (42.2%) were genotype D, 11 of them were males and 8 were females , 12\45 (26.6%) were genotype E and D co-infection 11 of them were males and 1 was female. The results obtained in the study were similar to those obtained by Mukhalid, *et al* in Sudan, where the rate of genotypes distribution of HBV was 46.0% D, 21.6% E, 18.9% A, and 13.5% D/E recombinant (Mukhalid, *et al*, 2014) this study was similar to our study because has same geographical distribution which both of them were in Sudan and HBV genotypes vary according to geographical distribution and individuals migration.

In comparing our findings with results from other countries we find**,** 87% of HBV/E and 13% of HBV/A in Côte d'Ivoire; 100% of HBV/E in Ghana; 67% of HBV/E and 33% of HBV/A in Cameroon; and 100% of HBV/A in Uganda (Joseph, *et al*, 2013) the result show high percentage of genotype E which was similar to our study and it's up to the common genotypes of HBV in African countries were E and Aa, D according to HBV genotypes distribution.

Also Ana, *et al* showed genotype A (55.3%), D (32%), F (5.3%), C (3.2%) and G (1%) (Ana, *et al*, 2014). Suggesting that a greater genetic diversity for this genotype than previously reported. Also study obtained by Mayte *et al* HBV-A was the most common (57%), followed by HBV-D (39%). HBV-A predominated among homosexual men (67%) while HBV-D predominated among intravenous drug users (67%) (Mayte, *et al*, 2004) this is depend on previous study was applied to know common genotype according to rout of transmission.

The genetic diversity of genotype E was found to be 0.37% for S gene and 1.7% for the complete genome, which represent the low diversity comparing with the other genotypes and sub-genotypes of HBV from 400 sequences of HBV West

African countries (Hubschen, *et al*, 2009). While genotype D shown to be the most divergent genotype after genotype C (Norder, *et al*, 2004).

The significant difference in intra group divergence of genotype E and D not seen in HBV\HIV co-infected patients, while seen in mono-infected patients HIV infection changes the heterogeneity of the virus lead to increase variability of HBV (Pal, *et al*, 2013).

There is deletion in pre-s1 and pre-s2 regions in genotype E while there is no deletion in genotype D among HBs-Ag positive HBV\HIV Co-infected patients, and there is deletion mutants found in genotype E and D isolated from Sudanese HBV monoinfected liver disease individuals this mutant deletion mainly was found in HCC patients with HBV genotype E while not found in genotype D patients, pre S deletion mutation affect the progression to serious liver disease in patients (Yousif, *et al*, 2015).

Severity of liver disease and the response of therapies in HBV vary according to HBV genotypes. The response to interferon in patients infected with genotype A and B is better than genotype C and D, and the response of lamivudine was better in genotype B and D than genotype A or C (Rajab and Asad, 2009).

In similar study by Anna *et al*, among 86/118 (72.9%) in Ghanaian HIV-positive patients with an HBV DNA sequence, the distribution of HBV genotypes was E in 82/86 (95.3%) and A in 4/86 (4.7%). Antiviral treatment status was not known for individual coinfected patients (Anna, *et al*, 2010). The risk of development of LMV resistant and the rate of hyperbilirubinemia, cirrhosis and HCC in HBV genotype B is higher than genotype C according to study applied in 96 HIV-HBV co-infection receiving LMV containing highly active antiviral therapy (Wang, *et al*, 2012). In recent study HBV genotype E occupies the majority of infection in

West Africa with a mix of genotypes and Asian\African variant of genotype A (Aa) and genotype D in North this describe the co-circulation of genotype E and D in liver disease patients and response to antiviral therapies. The availability of combination therapy, LMV and TDF containing HAART regimens need better understanding of unique African HBV genotypes (Jordan, *et al*, 2005).

5.2CONCLUSIONS

The findings of the present study disclosed a high percentage of HBV infection among HIV-positive patients, and the rate of HBV\HIV coinfecion in male was higher than female. Also the HBV genotypes among HIV in our study were genotype E and D and there is coinfection genotype E and D in some patients, genotype E give the highest percentage among the others genotype.

RECOMMENDATIONS

1. Further large surveillance should be carried out to study the common genotypes of HBV in other groups such as HCC, parental drugs users, multiple blood transfusion recipients and organ recipients to determine the true prevalence of HBV genotypes in Sudan is important.
2. Molecular detection methods, such as nested, RT-PCR, should be used to detect coinfection and lamivudine and tenofovir containing HAART regimens need to better understanding of unique Sudanese HBV genotypes.

6. References

1. Athena P. Kourtis, Marc Bulterys, Dale J. Hu, Denise J. (2015). HIV-HBV coinfection-A Global challenge. *PMC.* Engl J Med.5 (19): 17491752.
2. Anthony Tan, Sarene Koh, Antonio Bertoletti.(2015).Immune response in hepatitis B infection. *PMC.* 5(8): 421–428.
3. Ana Paula, Torres Santos, José Eduardo Levi. (2014). Identification of hepatitis B virus genotypes in the state of São Paulo. *SciELo*. 60(5): 417423.
4. Allan Ronald. (2005*).* the liver in HIV in Africa. *Antiviral Therapy*. 10(1):953–965.
5. Anupam Prakash. (2018). Hepatitis B virus transmission and health care workers: Epidemiology, pathogenesis and diagnosis. *Science direct*. 9(1).30-35.
6. Abigail E. Mitchell, Heather M. Colvin, R. Palmer Beasley. (2010). Institute of Medicine recommendations for the prevention and control of hepatitis B and C. *AASLD*. 51(3):729-733.
7. Anna Maria Geretti, Mauli Patel, Fred Stephen Sarfo, David Chadwick. (2010). Detection of Highly Prevalent Hepatitis B Virus Coinfection among HIV-Seropositive Persons in Ghana. *JCM* .48 (9) 3223-3230.
8. Bivigou-Mboumba B, Amougou-Atsama M, Zoa-Assoumou S. (2018) Hepatitis B infection among HIV infected individuals in Gabon. PLOS. 13(1): 590 -592.
9. Berthold Bivigou-Mboumba, Marie Amougou-Atsama, Samira Zoa-Assoumou. (2018). Hepatitis B infection among HIV infected individuals

in Gabon: Occult hepatitis B enhances HBV DNA prevalence. *PLOS*. 13(1): e0190592.

10. Bruss V. (2004). Envelopment of hepatitis B virus necleocapsid. *NCBI.* 106(2):199-209.
11. Beom Kyung Kim, Peter A Revill, Sang Hoon Ahn. (2011). HBV genotypes: relevance to natural history, pathogenesis and treatment of chronic hepatitis B. *Antiviral Therapy. (*16):1169-1186.
12. Bineeta, Kashyap,UrvashiTiwari, AnupamPrakash. (2018).Hepatitis B virus transmission and health care workers: Epidemiology, pathogenesis and diagnosis. Science direct. 9(1).30-35.
13. ChiahoShih, Ching-ChunYang, GansukhChoijilsuren, Chih-HsuChang. (2018).Heptititis B virus. *Science direct.* 26(4).p.386-387.
14. Center of Disease Control and prevention. (2012). Chapter 9 Hepatitis B Epidemiology and Prevention of Vaccine preventable disease. The pink Book: updated 12thEdition: 15-138.
15. Christian Trepo, MD, Prof Henery. (2014). Hepatitis B infection. *The lancet* .384 (9959):22053-2063.
16. Catherine MN Croagh, John S Lubel. (*2014).*Natural history of chronic hepatitis. *WJG.* 20(30): 10395-10404.
17. Chisari G and Ferari H. (1997). Hepatitis B virus infection: epidemiology and vaccination. *Epidemiologic Reviews*. 28(1): 112-125.
18. David Siebert and Stephen Locarnini. (1998). Hepatitis B: issues in laboratory diagnosis and vaccination. *NPS*. 21(3):72-5.
19. Frances V, Chisari, Carlo Ferrari, Mario U. (2004). Hepatitis B virus structure and biology. *Science Direct. 6(5):311-325.*

20. Francis V. Chisari, Masanori Isogawa, Stefan F. (2010). Pathogenesis of HBV infection. *PMC.* 58(4): 258–266.
21. Ferruccio B, Teerha P, Maurizia RB,Yun FL. (2010). Diagnostic markers of chronic hepatitis B infection and disease. *IMP.* 15(3):35-44.
22. Ganem D and Schneider RJ. (2001). Hepadnaviridea the viruses and their Replication in: knipe DM et al, eds. Fields Virology, 4thed .Philadelphia, Lippincott Williams and Wilkins. 2923-2969.
23. Guidotti LG and Chisari FV. (2006). Immunobiology and pathogenesis of viral hepatitis. *PubMed*.1 (6):23–61.
24. Gutam Ray. (2017). Current Scenario of Hepatitis B and Its Treatment in India. *PMC.* 5(3):227-296.
25. Heymann David L. (2008). Viral Hepatitis B. In: Control of Communicable Disease Manual 1^{9th} ed. *APHA*. 95(16) 284 -293.
26. Hollinger FB and Liang TJ. (2001). Hepatitis B virus. In: knipe DM *et al*. ,eds .Feilds Virology,4th ed. Philadelphia, Lipponcott Williams and wilkins, 2971-3036.
27. Howord.C.R. (1995). The structure of hepatitis B envelope and molecular variants of hepatitis B virus. JVH. 2(4):165-170.
28. Henry Bautista-Amorocho, Yeny Zulay Castellanos-Domínguez, Laura Andrea. (2014). Epidemiology, Risk Factors and Genotypes of HBV in HIV-Infected Patients in the Northeast Region of Colombia: High Prevalence of Occult Hepatitis B and F3 Subgenotype Dominance. *PLOS*. 9(12):e114272.
29. Hubschen JM, Mugabo J, Peltier CA, Karasi JC. (2009). Exceptional genetic variability of hepatitis B virus indicates that Rwanda is east of an emerging African genotype E/A1 divide. *PubMed*.81(3):435-40.

30.Joseph C. Forbi, Yousr Ben-Ayed,[a]Guo-liang Xia.(2013).

31.Jia-Horng, Kao, NanHuiW.(2000). Molecular characterisation of hepatitis B virus in HIV-1 subtype C infected patients in Botswana. *PMC. 2(9)*:335

32. Jordan J Feld, Ponsiano Ocama u, Pei-JerChen, Ming,YangLai. (2000). Hepatitis B genotypes and the response to interferon therapy. *Science Direct*. 33(6):998-1002.

33.Jennifer H, MacLachlan, Benjamin C. Cowie. (2015). Hepatitis B virus epidemiology. *PMC.* 5(5):a021410.

34.Jeong Eun Song, Do Young Kim. (2016). Diagnosis of hepatitis B. PMC. 4(18):338.

35.Jinlin Hou, Zhihua Liu, Fan GU. (2005). Epidemiology and Prevention of Hepatitis B Virus Infection. *PMC*. 2(1):50–57.

36.J.Y.N, LauMDT, L.WrightMD. (2003). Molecular virology and pathogenesis of hepatitis B. *science direct*. 342(8883):1311-1340.

37.Keeffe EB, Dieterich DT, Han SH. (2008). A treatment algorithm for the management of chronic hepatitis B virus infection in the United States. *CGH*. 6(12):1315-1341.

38.LievenStuyver, Sija De Gendt, Caroline Van Geyt, Fabien Zoulim, Michael Fried.(2000). Anew genotype of hepatitis B virus: complete genome and phylogenetic relatedness. *JGV*. 81:67-74.

39.Micheal Nassel, Heinz Schatter. (1993). Hepatitis B virus replication. *Science Direct. 1(6):221-228.*

40.Mustafa. (2014). Hepatitis B genotypes: Global virus distribution and clinical importance. PMC. 20(18): 5427–5434.

41. Mayte Pérez-Olmeda Marina Núñez Javier García-Samaniego. (2004). Distribution of Hepatitis B Virus Genotypes in HIV-Infected Patients with Chronic Hepatitis B: Therapeutic Implications. pubMed. 19(8):657-9.
42. Mahoney D and Kane R. (1999). Liver biopsy assessment in chronic viral hepatitis. *J Viral Hepat*. 14(1): 16-21.
43. Mel Krajden, Gail McNabb, Martin Petric, Can J. (2005). The laboratory diagnosis of hepatitis B virus. *PMC.* 16(2): 65–72.
44. Mahoney FJ. (1999). Update on diagnosis, management and prevention of hepatitis B virus infection Clin Microbial Rev. 12(2):351-366.
45. MukhlidYousif[a], HatimMudawi, WaleedHussein. (2014). Genotyping and virological characteristics of hepatitis B virus in HIV-infected individuals in Sudan. *Science direct.* 1(8): 125-132.
46. Norder H, Courouce IS, Coursaget P. (2004). Genetic diversity of hepatitis B virus strains derived worldwide: geno- types, subgenotypes, and HbsAg subtypes. PubMed. 47(6):289–309.
47. Naito, H, Hayashi, S, Abe, K. (2001).Rapid and specific genotyping system for hepatitis B virus corresponding to six major genotypes PCR using type-specific primers. JCM. 39,362-364.
48. Pal A, Panigrahi R, Biswas A. (2013). Influence of HIV- associated degree of immune suppression on molecular heterogeneity of hepatitis B virus among HIV co-infected patients. 436(1):134–42.
49. Robinson. (1999). Hepatitis B antigenic structure and different subtype distribution. *WJH*. 35(2):74-81.

50. Robert Flisiak, Waldemar Halota, Jerzy Jaroszewicz. (2017). Recommendations for the treatment of hepatitis B in 2017. *PMC*.3 (2):35-46.

51. Rajab A K and Asad A K. (2009). Hepatitis B virus Genotypes: Clinical & Therapeutic Implications. *PubMed*. 59(2):101-4.

52. Scott K, Fung Anna S, F. Lok M.D.(2007).Hepatitis B virus genotypes: Do they play a role in the outcome of HBV infection? *AASLD.* 40(4):7902.

53. Stefan H and Francis M. (2005). Diagnostic markers for chronic hepatitis B infection. *J Med Viro*. 64(5): 271–277.

54. Taylor and J. M. (2006). "Hepatitis delta virus". J.Virol. 344 (1): 71–6.

55. T. Jake Liang. (2010). Hepatitis B: The virus and disease. *PMC*. 49(5 Suppl): S13–S21.

56. Ueda K, T Tsurimoto, K MatsubaraJ. (1991). three envelope proteins of hepatitis B virus: large S, middle S, and major S proteins needed for the formation of Dane particles. *PMC*. 65(7):3521–3529.

57. Vanlandschoot.P, Cao T, Lerou-Reo G. (2003).The nucleocapsid of the hepatitis B virus: aremarkable immunogenic structure. *NCBI.* 60(20):6774.

58. Wolfram H Gerlich. (2013).Medical virology of hepatitis B: How it began and where we are now. *PMC* .10(2): 239.

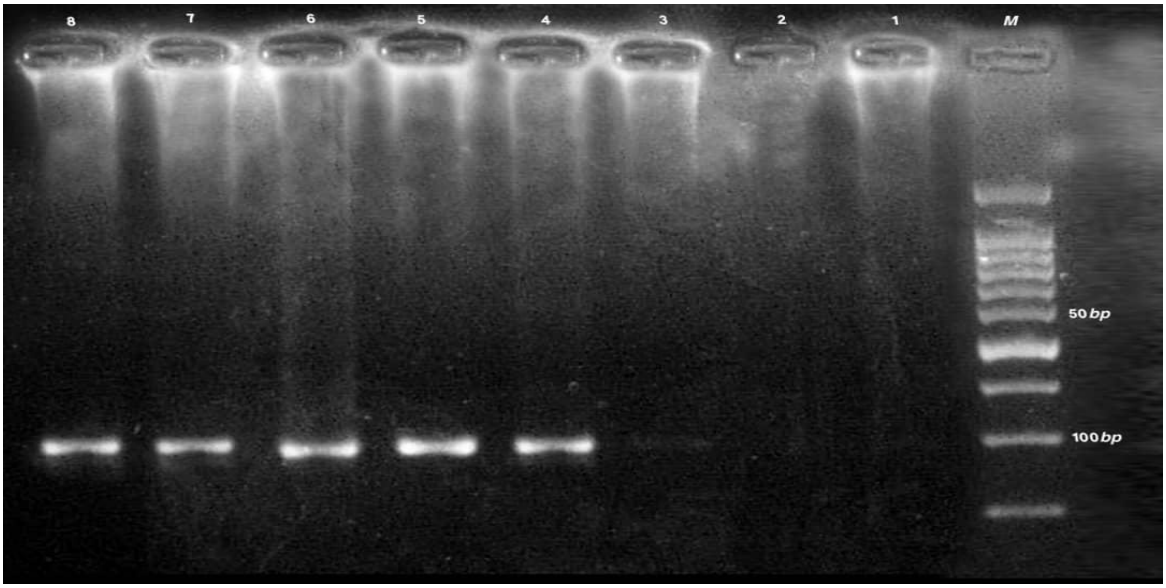
59. World Health Organization. (2018). Hepatitis B. *WHO*. 15(1):135.

60. Wang-Huei Sheng, Chien-Ching Hung, Sui-Yuan Chang. (2012). Differential Clinical and Virologic Impact of Hepatitis B Virus Genotypes B and C on HIV-Coinfected Patients Receiving Lamivudine-

Containing Highly Active Antiretroviral Therapy. *OXFORD.* 54(4):548555.

61. Xiaodong Zhang, Weiying Zhang, Lihong Ye. (2006).Pathogenesis of HBV hepatitis B infection. *Future medicine.* 1(1):144.
62. Yousif M, Mudawi H, Bakhiet S, Glebe D. (2009). Molecular characterization of hepatitis B virus in liver disease patients and asymptomatic carriers of the virus in Sudan. *BMC Infect Dis*.13(1)1:1328.
63. Yassin Elfaki, Enan KA, Elkhidir IM, Nagi AM. (2016). Molecular Detection of Hepatitis B Virus DNA in Human Immunodeficiency Virus Infected Patients in Khartoum, Sudan. *Mol Biol*. 6(1):2168-9547.

7. Appendix



(Figure 5) This figure show lane M marker lane 1,2 negative control lane 3,4,5,6 and 7 genotype D (119bp) lane 8 positive control positive for genotype D 119bp.