بسم الله الرحمن الرحيم

Republic of Sudan

Ministry of Higher Education and scientific Research

University of Shendi

Faculty of Graduate Studies and Scientific Research

Association of Interleukin-10 with different Clinical Stages of Hepatitis B Virus Infection in IbnSina Hospital-Khartoum State; 2018.

Dissertation submitted in Partial Fulfillment for the Requirements of M.Sc. Degree in Medical Laboratory Sciences (Microbiology)

By

Rania Mohammed Meskin Saied

(B.Sc. Of Shendi University.2010)

Supervisor

Dr. Waseem Sameer Kwami

(Assistant professor of microbiology, Shendi University)

2018

الآية

قال تعالي

﴿فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَى إِلَيْكَ وَحْيُهُ وَقُلْ رَبِّ زِدْنِي عِلْمًا﴾

[طه:114]

Dedication

To everyone who taught me how to be a valuable

member in community

Dear father

To the depth of longings and rhythm sympathy

Dear mother

To all my love and my life

My son

to crown of pleasure and secret of existence

Dear husband

To who bring happiness to my life

My sisters

To who make my life shining

My brothers

To my friends, And to everyone who smile on my face and help me

Rania

Acknowledgments

All thanks to Allah from the start to the end, and I wish to record my immense gratitude and appreciation to my supervisor **Dr. Waseem Sameer Kwami**, for his helpful suggestion and close supervision throughout the work and writing up the work

I am most grateful to my friend Dr**. Musa Abdalla** what can I say, Allah give you good things. Thank you for believing in me and for your limitless words of encouragement.

I would like to express endless thanks to Laboratory staff member of Ibnsina Hospital

Finaly I grateful to staff of Shendi University, especially Microbiology department as teachers, technician and workers

ABSTRACT

This is case control study conducted in Khartoum state in period from May to October (2018). The objective of the study was to determine the association of IL-10 with different clinical stages of HBV infection: acute, chronic, carriers, and cirrhotic.

A total of 90 participants were enrolled in this study; 60 participants were enrolled as case group and 30 participants were enrolled as control group, demographic and clinical data were collected using structured questionnaire. A venous blood was collected from all study participants and serum was harvested by centrifugation at 1000 rpm for 5 minutes, the serum level of ALT, AST were estimated spectrophotometrically, and IL-10 by ELISA technique according to manufactures instructions.

The level of IL-10, ALT, and AST was significantly elevated in case group when compared with control group, mean of IL-10(18.15ng/ml in case, 10.1ng/ml in control), mean of ALT (105.6u/l in case, 27.27u/l in control), and mean of AST(90.15u/l in case, 24.33u/l in control). The study revealed that, there was no significant statistically differance between the age and clinical stages of HBV infection (p.value 0.373),and sex and clinical stages of HBV infection (p.value 0.329).

The study concluded that the IL-10 level is higher in cirrhosis than the other clinical stages of HBV infections, and IL-10 can serve as significant marker for disease progression.

المستخلص

هذه الدراسة الوصفية أجريت في ولاية الخرطوم في الفترة من مايو إلى أكتوبر (2018).كان الهدف من الدراسة هو تحديد ارتباط IL-10 بمراحل التهاب الكبد البائي HBV المختلفة : الحاد ,المزمن, الناقل والتليف الكبدي.

تم جمع مجموعه 90 عينة في هذه الدراسة, منها 60 مريض كمجموعة دراسة و 30 عينة ضابطة وتم جمع البيانات السريرية باستخدام استبيان . ثم جمع عينات الدم من جميع المشاركين في الدراسة وتم فصلها عن طريق الطرد المركزي عند 1000 دورة في الدقيقة لمدة 5 دقائق ، وتم قياس مستوى ALT، AST و IL-10 تم استخدام طريقة الطيف الضوئي لقياس ALT و AST وتم قياس IL-10 بواسطة تقنية ELISA . واظهرت الدراسة ارتفاع ملحوظ في مستوي ALT، AST و IL-10 في حالة مجموعة الدراسة مقارنة مع مجموعة الضابطة .

واظهرت الدراسة ارتفاع ملحوظ في مستوي ALT, AST وIL-10 في وسط الحالة مقارنة مع المجموعة الضابطة. متوسط IL10(18.15ng/ml في الحالة. 10.1ng/mlفي المجموعة الضابطة).AST (في المجموعة الضابطة 24.33u/l. في مجموعة الحالة 90.15u/l). ومتوسط ALT(في المجموعة الضابطة 105.6u/l.في مجموعة الحالة 27.27u/l .

وكشفت الدراسة أنه لا يوجد ارتباط إحصائي مهم بين العمر والمراحل السريرية للعدوى(p.value 0.373) والجنس والمراحل السريرية للعدوى بفيروس التهاب الكبد البائي(p.value 0.323).

وخلصت الدراسة إلى أن مستوى IL-10 أعلى في تليف الكبد من المراحل السريرية الأخرى من العدوى بفيروس الالتهاب الكبدي البائي ، ويمكن أن يكون IL-10 بمثابة علامة هامة لتقدم المرض.

Table of Contents

List of Figures and Tables

List of abbreviations

Abbreviation	Full name
ACLF	acute-on-chronic liver failure
ALD	Alcoholic liver disease
ALT	Alaanine aminotransferase
Anti-HBs	Anti hepatitis B antibody
AST	Aspartate aminotransferase
ATG	Start codon
CccDNA	Covalently Closed Deoxy ribonucleic acid
CSIF	cytokine synthesis inhibitory factor
CTLs	Cytotoxic t lymphocytes
DNA	Deoxy ribonucleic acid
ELISA	Enzyme linked immune serpent assay
HBcAg	Hepatitis B virus core antigen
HBDNA	Hepatitis B virus deoxyribonucleic acid
HBeAg	Hepatitis B envelope antigen
HBIG	Hepatitis B immunoglobulin
HBs Ag	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HDV	Hepatitis D.
HRP	Horseradish peroxidase
IFN	Interferon
IL10	Interleukin 10
IL-10R	IL-10 receptor
IVF	In vitro fertilization

LCMV	Lymphocytic choriomeningitis virus
MDH	malate dehydrogenase
MGN	Membranous glomerulonephritis
MHC	Major Histocompatibility complex
NADH	Nicotinamide adenine dehydrogenase
NAFLD	Non-alcoholic fatty liver disease
NC	Normal control
NK	Natural killer cell
PBMCs	peripheral blood mono nuclear cells
PCR	Polymerase Chain Reaction
PDV	Plasma -derived vaccine
RcDNA	Relaxed Circular Deoxy ribonucleic acid
RgRNA	pregenomic RNA
RNA	Ribonucleic acid
RV	Recombinant vaccine
TLRs	Tool Like Receptor
TNF	Tumor necrosis factor
WHO	World Health Organization

CHAPTER ONE
INTRODUCTION AND OBJECTIVES

1.1. Introduction

Hepatitis means inflammation of hepatic cell which caused by many infectious agent (bacteria, virus and parasites) and noninfectious agent including alcohol and other poisoning. Viral hepatitis the most common cause of hepatitis (infectious agent) and mainly cause by hepatitis virus A, B, C, D, and E. Each of which is classified in a different virus family. These can be divided in to two groups, based on whether or not they can cause chronic infection. Those viruses that can do this routinely (hepatitis B and C virus) are particularly associated with chronic or late-stage liver disease and with the emergence of liver cancer. Hepatitis B virus (HBV) is a hepadnavirus that is transmitted variously by close contact, sexual activity, at birth also by blood transfusion. The hepatitis B is common cause of chronic viral hepatitis and may lead to complication such as liver cirrhosis and hepatocellular carcinoma (HCC).[1]

Hepatocellular carcinoma mean, liver cell carcinoma or hepatoma, one of malignant growth which worldwide spread and occur besides to as complication of viral hepatitis due to Alfa toxin and other causes that may lead to liver cirrhosis such as alcoholism. Particularly HBV infection related to HCC, because more than 85% of cases occur in countries with high rates of chronic HBV infection.[2] Hepatitis B virus is a noncytopathic virus; inflammation in the liver is mediated by host immune responses to the HBV-infected hepatocytes.[3]The world can be divided into three areas where the prevalence of chronic HBV infection is high (> 8%), intermediate (2-8%), and low (< 2%).[4, 5]

High endemicity areas include south-east Asia and the Pacific Basin (excluding Japan, Australia, and New Zealand), sub-Saharan Africa, the Amazon Basin, parts

of the Middle East, the central Asian Republics, and some countries in Eastern Europe. In these areas, about (70 to 90%) of the population becomes HBV infected before the age of 40, and (8 to 20%) of people are HBV carriers. [6]

In countries such as China, Senegal, Thailand, infection rates are very high in infants, and continue through early childhood. At that stage, the prevalence of HBsAg in serum may exceed 25%. In other countries such as Panama, Papua New Guinea, Solomon Islands, Greenland, and in populations such as Alaskan Indians, infection rates in infants are relatively low and increase rapidly during early childhood.[6]

Low endemicity areas include North America, western and Northern Europe, Australia, and parts of South America, The carrier rate here is less than (2%), and less than (20)% of the population is infected with HBV.[6,4]

The immune system interacts with HBV with many different mechanisms in order to elimination and clearance of the virus, this can be humeral with specific antibody or cell mediated immunity,[7] after the virus enter the hepatic cell, host response initiated, dendritic cells processed the virus in the hepatic cell, the dendritic cells recognize virus by toll-like receptor (TLRs) and then presented to the immune system cells through the major histocompatibility complex MHC-1 and MHC-2.[8]

Clinical outcome showed significant difference, the role of cytokines attracted more and more attention. when people were infected with HBV the immune cells secreted large amount of cytokines, which may cause the virus to clear or persistent infection or even liver damage in different outcome.[9] Persistent hepatitis B viral infection results in chronic hepatitis, liver cirrhosis and hepatocellular carcinoma.[10] The persistence of infection was directly correlated with insufficient and ineffective both of $CD4^+$ and $CD8^+$ T Cells.[7]

Interleukin-10 (IL-10) also known as cytokine synthesis inhibitory factor (CSIF), is the charter member of the IL-10 α-helical cytokine family that also includes IL-19, IL-20, IL-22, IL-24, and IL-26/AK155.[11,12]

IL-10 is secreted by many activated hematopoietic cell types as well as hepatic stellate cells, keratinocytes, and placental cytotrophoblasts. Whereas human IL-10 is active on mouse cells, mouse IL-10 does not act on human cells.[13,14]Mature human IL-10 shares (86%)amino acid sequence identity with equine IL-10 and (72% - 80%) with bovine, canine, feline, guinea pig, mouse, ovine, porcine, and rat IL-10.It contains two intrachain disulfide bridges and is expressed as a 36 Kd a non-covalently-associated homodimer.[13, 15, 16]

IL-10 mediates its biological activities through a heteromeric receptor complex composed of the type II cytokine receptor subunits IL-10 Rα and IL-10 Rβ. IL-10 Rα is a 110 kD a trans- membrane glycoprotein that is expressed on lymphocytes, NK cells, macrophages, monocytes, astrocytes, intestinal epithelial cells, cytotrophoblasts, and activated hepatic stellate cells,[17,18] while the 75 kD atransmembrane IL-10 Rβ is widely expressed.[19, 20]

The IL-10 dimer binds to two IL-10 Rα chains, triggering recruitment of two IL-10 Rβ chains.[19, 20] IL-10 Rβ does not bind IL-10 directly but is required for signal transduction, IL-10 Rβ also associates with IL-20 Rα, IL-22 Rα1, or IL-28 Rα to form the receptor complexes for IL-22, IL-26, 28, and IL 29.[21,22]

2.2. Rationale

WHO estimated than about 240 million individuals are chronically infected with HBV and more than 780.000 people die every year due to complications of hepatitis B, including cirrhosis and liver cancer.[23, 24]

The high spread numbers of HBV infected patients and the high incidence of HCC among them lead to more focusing of pathogenesis of the virus and the way through which the cancer begin and develop.

Also there is few authentic data regarding to the relation of IL10 with prognosis of HBV and development of cirrhosis, the current study is conducted to determine the association of IL-10 with different stages of HBV infection.

1.3. Objectives:

1.3.1. General objective:

To determine the association of IL-10 with different stages of HBV infection.

1.3.2. Specific objectives:

1. To measure the level of IL-10, AST, and ALT in case and control groups.
2. To correlate between the level of IL-10, AST, ALT among sex and age.
3. To correlate between the level of IL-10, AST, ALT and different clinical stages of HBV infection.

CHAPTER TWO

LITERATURE REVIEW

2.1. Virologic Characteristics of hepatitis B virus

Hepatitis B virus (HBV) is prototype, small, icosahedral enveloped, hepatotropic virus with a partly double strand, relaxed circular DNA genome. It's member of the hepadnaviridae family.[25]

Based on sequence comparison HBV is classified in to eight genotypes, A to H. Three types of viral particles are visualized in infectious serum by electron microscopy, spherical particles 20nm,filamentous particles 22nm, and Dane particles 42nm (virion).[26]

All these particles have common HBsAg on their surface. The spherical and filamentous particles are composed of HBsAg and host-derived lipid without HBV genome, thus they are noninfectious, but Dane particles composed of a lipid envelope containing HBsAg that surround an inner nucleocapsid composed of hepatitis B core antigen complexed with virally encoded polymerase and the viral DNA genome.[27],[28]

2.2. Replication of HBV

Replication of the hepadnaviral genome can done by steps,infectiousvirions contain in their inner icosahedral core, the genome as a partially double-stranded, circular but not covalently closed DNA of about 3.2kb in length(relaxed circular or Rc-DNA), upon infection, the Rc-DNA is converted inside the host cell nucleus into a plasmid-like covalently closed DNA(cccDNA),from the cccDNA, several genomic and sub- genomic RNAs are transcribed by cellular RNA polymerase II,of these the pregenomic RNA (RgRNA) is selectively packaged in to progeny capsids and is reverse transcribed by the co-packaged P protein into new Rc-DNA

genome, mature Rc-DNA containing, but not immature RNA containing. Nucleocapside can be used for intracellular cccDNA amplification or be enveloped and released from the cell as progeny virions.[29]

2.3. Genome of hepatitis B virus

The genome of HBV is made of circular DNA, but it is unusual because the DNA is not fully double-stranded. One end of the full length strand is linked to the viral DNA polymerase. The genome is 3020–3320 nucleotides long (for the full-length strand) and 1700–2800 nucleotides long (for the short length-strand).[30]

The negative-sense (non-coding) is complementary to the viral mRNA. The viral DNA is found in the nucleus soon after infection of the cell. The partially double-stranded DNA is rendered fully double-stranded by completion of the (+) sense strand and removal of a protein molecule from the (−) sense strand and a short sequence of RNA from the (+) sense strand. Non-coding bases are removed from the ends of the (−) sense strand and the ends are rejoined. There are four known genes encoded by the genome, called C, X, P, and S. The core protein is coded for by gene C (HBcAg), and its start codon is preceded by an upstream in-frame AUG start codon from which the pre-core protein is produced. HBeAg is produced by proteolytic processing of the pre-core protein. In some rare strains of the virus known as Hepatitis B virus precore mutants, no HBeAg is present.[31]

The DNA polymerase is encoded by gene P. Gene S is the gene that codes for the surface antigen (HBsAg). The HBsAg gene is one long open reading frame but contains three in frame "start" (ATG) codons that divide the gene into three sections, pre-S1, pre-S2, and S. Because of the multiple start codons, polypeptides of three different sizes called large (the order from surface to the inside: pre-S1, pre-S2, and S), middle (pre-S2, S), and small (S) are produced.[32]

The function of the protein coded for by gene X is not fully understood but it is associated with the development of liver cancer. It stimulates genes that promote cell growth and inactivates growth regulating molecules.[33]

2.4. Serotypes and genotypes:

The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes presented on its envelope proteins, and into eight major genotypes (A–H). The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus. Differences between genotypes affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination.[34,35]

There are two other genotypes I and J but they are not universally accepted as of 2015,[36] Genotypes differ by at least (8%) of their sequence and were first reported in 1988 when \six were initially described (A–F).[37] Two further types have since been described (G and H),[38] most genotypes are now divided into sub genotypes with distinct properties.[39]

2.5. Epidemiology of HBV:

Approximately 350 million people worldwide are chronically infected with HBV. Nearly one in three person has been exposed to HBV, making it one of the most common chronic viral infection in the world. In the United State 125 million people are chronically infected, with the majority being foreign born. Eastern and Southern Asia as well as sub-saharah Africa, are endemic regions.[40]

Sudan is classified among the countries with high hepatitis B seroprevalence. Exposure to the virus varied from (47%-78%), with hepatitis B surface antigen prevalence ranging from (68%) in central Sudan to(26%) in southern Sudan.[41]

2.6. Transmission of HBV:

HBV is transmitted by percutaneous and mucous membrane exposure to infectious blood and body fluids that contain blood (semen, serum and saliva). Percutaneous exposure that have resulted in HBV transmission include transfusion of blood or blood products, contaminated equipment used for therapeutic injection and other health-care related procedures, illegal injection drug use and needle sticks or other injuries from sharp instruments sustained by hospital personnel and tattooing, Perinatal and sexual transmission of HBV usually results from mucous membrane exposures to infectious blood or serum -derived body fluids no infection have been demonstrated in susceptible persons orally exposed to HBsAg positive saliva.[42]

2.7. Pathogenesis of HBV:

After the virus enters the hepatic cells, the immune system interacts with HBV with many different mechanisms in order to eliminate and clear the virus. Defense against HBV by adaptive immune response, either humeral antibody response contributes to clearance of circulating virus particles and the prevention of viral spread with in the host while the cellular response eliminate infected cells by cytotoxic T lymphocyte (CTL). Also cytotoxic T lymphocyte inhibits HBV gene expression through the secretion of antiviral cytokines and these cytokines clear the virus during HBV infection.[43, 44]

2.8. Mechanisms of host immune response:

Hepatitis B virus primarily interferes with the functions of the liver by replicating in hepatocytes.

A functional receptor is NTCP,[45] there is evidence that the receptor in the closely related duck hepatitis B virus is carboxy peptidase D.[46,47] The virions bind to the host cell via the pre S domain of the viral surface antigen and are subsequently internalized by endocytosis. HBV-preS-specific receptors are expressed primarily

on hepatocytes; however, viral DNA and proteins have also been detected in extra hepatic sites, suggesting that cellular receptors for HBV may also exist on extra hepatic cells.[48]

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.[49]

Although liver damage is initiated and mediated by the CTLs, antigen-nonspecific inflammatory cells can worsen CTL induced immunopathology, and platelets activated at the site of infection may facilitate the accumulation of CTLs in the liver.[50]

2.9. Clinical Feature of HBV

2.9.1. Acute Hepatitis B

Acute infection with hepatitis B virus is associated with acute viral hepatitis, an illness that begins with general ill-health, loss of appetite, nausea, vomiting, body aches, mild fever, and dark urine, and then progresses to development of jaundice. It has been noted that itchy skin has been an indication as a possible symptom of all hepatitis virus types. The illness lasts for a few 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.[51]

Most acute hepatitis infection patients have mild asymptomatic and subclinical illness undetected. Some acute HBV infection develop clinical symptoms and sign

of hepatitis, range from mild symptoms of fatigue and nausea to more marked symptoms and jaundice. The incubation period of acute HBV range from 1-6monthes (averages 2-3monthes) after exposure. During this phase serum alanine transferees (ALT) level rise and high level of HBsAg and HBV DNA, also detect anti-HBcIgM.[51]

2.9.2.Chronic Hepatitis B

Chronic infection with hepatitis B virus either may be asymptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis over a period of several years. This type of infection dramatically increases the incidence of hepatocellular carcinoma (HCC; liver cancer). Across Europe, hepatitis B and C cause approximately 50% of hepatocellular carcinomas.[52,53]

The chronic hepatitis is asymptomatic or has nonspecific symptoms (arthralgia). Chronic hepatitis characterized by the persistence of serum HBsAg for more than 6 months. In chronic hepatitis found HBsAg and HBV DNA in high titers and serum aminotransferase level mild to moderate elevation.[51] Chronic HBV carriers have an aggravation of their symptom include nausea, loss of appetite and jaundice, an acute exacerbation of disease should be considered. In HBV carriers patients, liver disease progresses to HCC through cirrhosis.[51] Chronic carriers are encouraged to avoid consuming alcohol as it increases their risk for cirrhosis and liver cancer. Hepatitis B virus has been linked to the development of membranous glomerulonephritis (MGN).[54] Symptoms outside of the liver are present in (1–10%) of HBV-infected people and include serum sickness like syndrome, acute necrotizing vasculitis (polyarteritisnodosa), membranous glomerulonephritis, and papular acrodermatitis of childhood (Gianotti–Crosti syndrome).[55,56]The serum-sickness–like syndrome occurs in the setting of acute hepatitis B, often preceding the onset

of jaundice.[57] 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 hepatitisB.[43] About (30–50%) of people with acute necrotizing vasculitis (polyarteritisnodosa) are HBV carriers.[58]

HBV-associated nephropathy has been described in adults but is more common in children Membranous glomerulonephritis is the most common form,[59] other immune-mediated hematological 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.[60]

2.10. Hepatocellular carcinoma (HCC):

Hepatocellular carcinoma or hepatoma accounts for more than 90% of all cases of primary liver cancer.[61] It is the sixth most common type of cancer worldwide and has shown a significant increase in its incidence, becoming third leading cause of cancer-related mortality.[62]

2.10.1.Etiology of HCC:

Cirrhosis, defined as fibrosis associated with nodular regeneration is considered a premalignant condition.[61] In Western countries, including Brazil, (70-80%) of HCC cases are associated to cirrhosis secondary to chronic infection with either hepatitis B or C viruses.[63] Alcohol also is an important predisposing factor to cirrhosis and HCC. In virtually all cases of HCC associated with the presence of HBV, there is integration of the HBV genome into the hepatocyte DNA.[61] In addition to that there are patients with negative serology for B-virus and presence of HBV in the tumor Non-alcoholic steatohepatitis is a risk factor for liver cirrhosis and HCC especially in obese patients, other risk factors for the onset are

aflatoxins and metabolic diseases, such as hemochromatosis, type I glycogenesis, alpha-1-antitrypsin deficiency, Wilson's disease and porphyria's. HCC can rarely occur without recognized risk factors. Fibro lamellar type, for example, is most often unrelated to previous cirrhosis or viral liver disease.[64]

2.10.2. Pathology of HCC:

HCC may present as a unifocal, multifocal, or diffusely infiltrative tumor.[61] All patterns demonstrate broad potential for vascular invasion. When associated with cirrhosis, HCC usually arises from malignant transformation of a regenerative nodule. There is stimulation to angiogenesis, and the tumor receives abundant arterial vascularization.[65] The mean tumor duplication time is about 200 days this time decreases as tumor increases. With up to 3 cm in size, HCC is generally well differentiated, encapsulated, and has low potential for blood vessel invasion. When it reaches approximately 5 cm in size, the nodule begins to lose differentiation and to exhibit microscopic vascular invasion acquiring capacity to generate metastases. [66,67,68]

2.11. Serum aminotransferases (ALT, AST):

Hepatocellular injury, whether acute or chronic, results in an increase in serum concentrations of aminotransferases. Alanine aminotransferase (ALT) originates primarily from the hepatocytes, whereas aspartate aminotransferase (AST) is found additionally in the heart, skeletal muscle tissue, kidney and brain. As a consequence, serum AST may also increase in response to pathological processes in the heart or skeletal muscle,[69] while serum ALT is considered a fairly specific marker of liver disease. Elevated serum aminotransferase levels can be found in asymptomatic patients for a variety of reasons, e.g. excessive alcohol intake, overweight, viral or autoimmune hepatitis, hemochromatosis, Wilson's disease, Į1-antitrypsin deficiency, coeliac spur, genetic disorders in muscle metabolism,

acquired muscle diseases, or strenuous exercise.[70] When interpreted together, aminotransferases can provide useful information on the etiology of liver disease, an elevation in the enzyme activity ratio AST/ALT, for example, having been considered suggestive of an alcoholic etiology.[71, 72] However, a recent study by Kotronen et al. (2010) reported similar enzyme ratios in the course of diseases when comparing Alcoholic liver disease(ALD) and no-alcoholic fatty liver disease (NAFLD). It has been suggested that a drinking-related deficiency in pyridoxal 5'-phosphate (vitamin B6, an active enzyme cofactor) in patients with more advanced liver disease may reduce ALT serum activity and contribute to the increase in the AST/ALT ratio that is frequently observed in alcoholic patients.[73] It has also been suggested that alterations in the relative activities of AST and ALT may be related to the occurrence of hepatic mitochondrial damage and skeletal or cardiac muscle injury (alcoholic myopathy), which are common among alcoholic patients.[70]

2.12. Laboratory Diagnosis of HBV infection

2.12.1. Serological Diagnosis

The following laboratory test may be used to assess various stage of hepatitis B virus infection:

- Hepatitis B surface antigen (HBsAg).
- Hepatitis B envelope antigen (HBeAg).
- Hepatitis B core antibody immunoglobin M (anti HBcIgM).
- Hepatitis B core antibody immunoglobin G (anti HBcIgG).
- Hepatitis B virus deoxyribonucleic acid (HBV DNA).

Table (2.12.1.1) Interpretations of Hepatitis B Serologic Test Results [74]

Marker	Result	Interpretation
HBsAg Anti-HBc Anti-HBs	Negative Negative Negative	Susceptible
HBsAg Anti-HBc Anti-HBs	Negative positive positive	Immune due to natural infection
HBsAg Anti-HBc Anti-HBs	negative negative positive	Immune due to hepatitis B vaccination
HBsAg Anti-HBc IgMAnti-HBc Anti-HBs	positive positive positive negative	Acutely infected
HBsAg Anti-HBc IgM Anti-HBc Anti-HBs	Positive Positive Negative Negative	Chronically infected
HBsAg Anti-HBc Anti-HBs	Negative Positive Negative	Interpretation unclear 1-resolved infection 2-false-positive anti-HBc,thus susceptible 3-lowlevelchronic infection 4-resolving acute infection

2.12.2. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA for detection of hepatitis B marker, simple performed, designed to read by eye, was sensitive. In this method antibody or antigen bind to solid surface and enzymes system linked to the complex, the remaining enzymes were wash and extend of enzyme activity is measured. This gives indication that antibody or antigen are present in test specimen.[75,76]

2.12.3. Polymerase Chain Reaction (PCR)

The polymerase chain reaction technique is a method for amplifying nucleic acid by repeated cycle of high temperature, first step template denaturation, oligonucleotides primer hybridization second step and polymerase extension third step. Used thermo stable polymerase Tag (thermos aquaticus) because they do not loss the enzymatic activity at high temperature. The result of amplification combined with agarose gel electrophoresis and southern blot hybridization analysis, to detect small quantities of hepatitis B virus DNA in serum of patient with hepatitis.[77]

2.12.4. Detection of Hepatitis B Virus in Tissue Specimen

The sequences of hepatitis B virus in liver tissue are usually analyzed and determined by the technique of DNA extraction from a liver specimen, gel electrophoresis, southern blotting and molecular hybridization with radioactive labeled HBV DNA probe, The method is not suitable for routine clinical investigation .In this method using HBV DNA probe to detect the HBV DNA sequences in formalin fixed liver biopsy specimen of chronic liver disease. The result compared with HBV serologic marker and with the liver histopathologic feature, in presence of ground glass cells or shikate staining positivity.[78]

2.13. Treatment of HBV infection

The main aim of treatment of chronic hepatitis B virus is to suppress HBV replication and to induce mitigation of liver disease before development of cirrhosis and hepatocellular carcinoma.

Two approaches are used Interferon therapy used to induce permanent immune control of infection via stimulation of the hosts antiviral immune response. Is most successful during the immune clearance phase of the infection, Nucleoside analog therapy is to block viral DNA synthesis and thereby reduce the number of infected hepatocytes.[79,80]

2.14. Prognosis of HBV infection

Hepatitis B virus infection may be either acute (self-limiting) or chronic (long-standing). Persons with self-limiting infection clear the infection spontaneously within weeks to months. Children are less likely than adults to clear the infection. More than 95% of people who become infected as adults or older children will stage a full recovery and develop protective immunity to the virus. However, these drops to (30%) for younger children, and only (5%) of newborns that acquire the infection from their mother at birth will clear the infection.[81] This population has a (40%) lifetime risk of death from cirrhosis or hepatocellular carcinoma of those infected between the age of one to six, (70%) will clear the infection.[82,83] Hepatitis D (HDV) can occur only with a concomitant hepatitis B infection, because HDV uses the HBV surface antigen to form a capsid,[84] Co-infection with hepatitis D increases the risk of liver cirrhosis and liver cancer.[85]

2.15. Prevention of HBV infection

2.15.1 Vaccine:

Vaccines for the prevention of hepatitis B have been routinely recommended for babies since 1991 in the United States.[86]

The first dose is generally recommended within a day of birth.[87] Most vaccines are given in three doses over a course of months. A protective response to the vaccine is defined as an anti-HBs antibody concentration of at least 10 mIU/ml in the recipient's serum. The vaccine is more effective in children and 95 percent of those vaccinated have protective levels of antibody. This drops to around (90%) at 40 years of age and to around 75 percent in those over 60 years. The protection afforded by vaccination is long lasting even after antibody levels fall below 10 mIU/ml. For newborns of HBsAg-positive mothers: hepatitis B vaccine alone, hepatitis B immunoglobulin alone, or the combination of vaccine plus hepatitis B immunoglobulin, all prevent hepatitis B occurrence.

Furthermore, the combination of vaccine plus hepatitis B immunoglobulin is superior to vaccine alone,[88] this combination prevents HBV transmission around the time of birth in (86%) to (99%) of cases.[89] Tenofovir given in the second or third trimester can reduce the risk of mother to child transmission by (77%) when combined with hepatitis B immunoglobulin and the hepatitis B vaccine, especially for pregnant women with high hepatitis B virus DNA levels.[90] However, there is no sufficient evidence that the administration of hepatitis B immunoglobulin alone during pregnancy, might reduce transmission rates to the newborn infant.[91] No randomized control trial has been conducted to assess the effects of hepatitis B vaccine during pregnancy for preventing infant infection, All those with a risk of exposure to body fluids such as blood should be vaccinated, if not already. Testing to verify effective immunization is recommended and further doses of vaccine are given to those who are not sufficiently immunized.[92]

Both types of the hepatitis B vaccine, the plasma-derived vaccine(PDV) and recombinant vaccine (RV) are of similar effectiveness in preventing the infection in both healthcare workers and chronic renal failure groups With one difference noticed among health worker group, that the RV intramuscular route is

significantly more effective compared with RV intradermal route of administration.[93]

2.15.2 Other:

In assisted reproductive technology, sperm washing is not necessary for males with hepatitis B to prevent transmission, unless the female partner has not been effectively vaccinated. In females with hepatitis B, the risk of transmission from mother to child with IVF is no different from the risk in spontaneous conception.[94] Those at high risk of infection should be tested as there is effective treatment for those who have the disease, Groups that screening is recommended for include those who have not been vaccinated and one of the following: people from areas of the world where hepatitis B occurs in more than (2%), those with HIV, intravenous drug users, men who have sex with men, and those who live with someone with hepatitis B.[95]

2.16. Cytokines

Some cytokines secreted by activation of these cells, and immunologic response designed according to them, cytokines are small soluble protein secreted by immune system and body cell, They act as part of intracellular interaction in immune system. These cytokines are bound to their specific cellular receptor, the function by autocrine or paracrine effects and may induce or inhibit cytokines regulating genes, Recently,100 different cytokines have been reported, and they are classified according to their role, These protein play a key role in polarization, directing and regulation of the immune response.[8]

2.16.1. Anti-Inflammatory Cytokines

Are a series of immune regulatory molecules that control the pro-inflammatory cytokines response. Cytokines act in concert with specific cytokines inhibitors and

soluble cytokines receptor to regulate the human immune response. The major anti-inflammatory cytokines include IL-10, IL-4, IL-11,IL-6 and IL-10.[96]

2.16.2. Interleukin-10

The most important cytokines is interleukin-10(IL-10),Is a type II cytokines, has old name cytokines synthesis inhibitor factor (CSIF), it was first define in 1989 as the cytokines synthesis inhibitor. In human IL-10 is produce mainly by monocytes, T cell, B cell, macrophage and dendritic cell, also observed in keratinocytes of the skin, epithelial cell and some tumor cells.[97]

2.16.2.1. Function of IL-10

The main biological function of IL -10 are to regulate or decrease the inflammatory response produce by dendritic cells, macrophage and reducing the adaptive responses of CD4Tcell, the expression of the major histocompatibility complex class II and the accessory co-stimulatory molecules CD80 and CD86 by dendritic cells are reduce due to cytokines because is a potent inhibitor, in general the effect is to inhibit the maturation of these cells.[97] Inhibition of dendritic cell maturation causes a reduction of the pro-inflammatory cytokines interferon gamma(IFNY), IL-5and IL-4 from T. cell. Also it inhibits the production of other inflammatory mediator tumor necrosis factor (TNF) and IL-10. On naïve CD4 T cell, IL -10 inhibit CD28 signaling, this lead to unable to activate, IL-10 stimulate NK Cell proliferation and promote B-cell activation, IL-10 act on IL-10 receptor after secreted.[97]

2.16.2.2. Role of IL-10 in Infection

Initial studies revealed that deficiencies in IL-10 through disruption of the IL-10 gene on IL-10 signaling via antibody blockade of the IL-10 receptor (IL-10R) that the majority of intercellular infection are controlled better or cleared faster in the absence of IL-10. Abrogation of IL-10 signaling leads to enhanced survival after

infection and is associated with enhanced adaptive immune response, including CD4Tcell IFNγ production and sustained production of the pro-inflammatory milieu. The absence of IL-10 is often initially beneficial to the host. Enhanced and prolonged production of inflammatory cytokines can lead to septic shock in the context of viral, bacteria or fungal infection. Because inflammatory molecules can often be potent activators of cell death, increasing level of I IL-10 can moderate the extent of apoptosis that is induced in response to infection.[98]Excessive IL-10 production can inhibit pro-inflammatory response to a number of pathogen to extent that pathogen can escape immune control, resulting in either fulminant and rapidly fatal or chronic non healing infection conversely, ablation of IL-10 signaling during normally benign infection may increase pro-inflammatory response by enhancing pathogen control at the considerable cost of more sever immunopathology importantly it is often not clear whether elevated concentration of IL-10 during virulent infection are accuse or a consequence of high pathogen burdens the resolution of infection requires a coordinated response in which initial pro-inflammatory mechanisms clear the pathogen and are subsequently limited by IL-10 before pathology occurs.[98]

2.16.2.3. Role of IL-10 in virus infection

Certain pathogen have evolved strategies to escape deletion or overcome immune response, rendering them capable of persisting in the host, these pre-dominantly include viruses. Persistence of HBV due to potential contributing factors include mutational escape which lead to inactivation of B-cell and T-cell epitopes e.g. HBeAg has been shown to suppress the antibody and T-cell response to HBcAg in adult T. cell receptor transgenic mice. HBsAg suppress immune elimination of infected cells by functioning as high dose tolerogen can be observed IL-10 production increase systemic. Elevated IL-10 signaling can inhibit pro-inflammatory cytokine production through direct targeting of immune effector

types, but also indirect modulate immune function by preventing maturation of macrophage and dendritic cell, thereby limiting co-stimulator, antigen presentation, and chemokine secretion capacity of the host interestingly, a number of viruses are capable of expressing IL-10 homologs that often bear strong sequence homology with host cellular IL-10.[98] Thus it has been proposed that IL-10 may play a role in maintaining persistence and pathogenicity in chronic infection Proof of this concept is best demonstrated in a model of chronic viral infection using LCMV clone. Here systemic IL-10 production coincides with a loss of CTL response directed against the virus. Neutralizing IL-10 signaling either through receptor antibody blocked or specific gene deletion led to rapid resolution of persistent LCMV clone. Thus in this case, systemic anti-inflammatory response rather than acute deficiencies in antiviral response elicited by LCMV lead to chronic viral infection.[33]

Etiologically, carcinogenesis of HCC is a complex multistep and multifactor process, in which many factors are implicated as we know; chronic infection with hepatitis B virus is the most well established environmental risk factor for HCC worldwide. However, only a fraction of HBsAg carriers eventually develop HCC later in life the exact mechanism of hepatocarcinogenesis is still incompletely understood, and the risk factor for HCC still need to be further elucidated.[99]

2.17. Previous studies

A study conducted in China by Wang, *et al* revealed that the IL-4, IL-12 p70 and IFN-γ were undetectable; IL-1β, IL-6, IL-8, IL-10 and TNF-α levels were significantly higher than in NC. Moreover, cytokines reached the highest levels in acute exacerbation of CHB, with the exception of IL-2 and IL-8. When comparing the HBV-ACLF patients prior to and at the time of acute-on-chronic liver failure (ACLF) diagnosis, IL-10 was the only cytokine that exhibited a significant

decrease (P = 0.008). IL-10 concentrations were positively correlated to ALT levels (r = 0.711, P < 0.001).[100]

A study conducted in West India, by Ozguler Metal, revealed that the result of Interleukin-10 levels of 25 patients with hepatitis B virus (HBV) DNA levels between 2000 and 20 000 IU/mL were compared with those of 25 subjects in the control group, and the level in the chronic hepatitis B group was statistically significantly higher ($p < 0.05$). Interleukin-10 levels of 38 patients with HBV DNA > 20 000 IU/mL were statistically significantly higher than those in the control group. When chronic hepatitis B patients were compared among themselves, IL-10 levels increased as HBV DNA levels increased. Also, when IL-10 levels of hepatitis B 'e' antigen (HBeAg) positive patients were compared with those of HBe Ag negative patients, the difference was not statistically significant.[101]

A study conducted in India, by Roli,Saxena, etal, revealed that the IL-10 protein and mRNA levels in peripheral blood mono nuclear cells (PBMCs) showed a significant elevation as the disease progressed to cirrhosis. But, no variation was observed in the IL-10 levels in subjects with different IL-10 genotype.[102]

A study conducted in India, by Fazal, *et al* revealed that IL-10 is elevated more in chronic hepatitis B with positive HBeAg and raised ALT in comparison to asymptomatic carrier, resolved acute hepatitis B and control, the HBe antigen may be responsible for the raised IL-10 levels.[103]

A study conducted in China by Bozkaya H, *et al* revealed that the level of IL-4 was significantly lower in patients in comparison with the controls (P < 0.05). However, the level of IL-10 was not significantly different in patients and healthy individuals (P > 0.05). The concentration of IFN-γ was significantly higher in patients compared with the healthy controls (P < 0.05). No significant correlation

was found between serum levels of IL-4, IL-10 and IFN-γ and nor was it found between the levels of these cytokines and serum ALT level.[104]

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

This is an analytical case control study.

3.2 Study area

The study was conducted in IbnSina hospital in, Khartoum state, Sudan, Mohammed Nagib street, contain 10 units, and average of frequency about 1000 patient's daily.

3.3 Study duration

The study was conducted during the period from April to October 2018.

3.4 Study population

The study population comprised of 60 known hepatitis B patients unselected for age of onset or family history and control sample consist of 30 healthy participants free from hepatitis B infection.

3.5 Inclusion criteria

Control group were HBV negative, case group were positive for HBsAg screened by ELISA, cirrhotic group determined by physical examination and abdominal ultra sound, Acute group determined by symptoms considering recent infection by HBV, and carriers were determined by using serological markers (positive anti core& negative anti envelope).

3.6 Exclusion criteria

Case group exclude individuals with no hepatitis B infection, or suffering from hepatitis due to other causes than HBV. The participants were not recruited as

control group if they were HBV positive or diagnosed with liver cirrhosis, HCC or any other known type of cancer.

3.7 Sample size

A total of 90 subjects were enrolled in this study. Control group includes 30 healthy subjects and hepatitis B case group include 60 hepatitis B positive subjects.

3.8 Data collection:

An interview with structured questionnaire was done for all participants in this study for obtaining the demographic and clinical data.

3.9. Data analysis:

Data were analyzed by statistical package for social sciences (SPSS) version 23.

3.10. Study procedure:

3.10.1. Collection of blood sample:

A 5 ml of venous blood was collected from all study participants, a soft tubing tourniquet was applied on arm of the patient to enable the veins to be seen and felt, then puncture site was cleaned with 70% ethanol and allowed to dry,10 ml capacity anticoagulant-free evacuated tube collection systems was used to collected blood sample, the container has a vacuum which is used to draw the blood into the container, then inter one end of the needle is situated in the patient's vein and the other end through the cap of the container, Bleeding from the venipuncture site was checked and stopped, the evacuated tube was stand until clotting of the blood the centrifuge it at 1000 rpm for 5 minutes, the clear serum was collected in other sterile container and preserved at minus 20ºC for further investigations.[105]

3.10.2. ELISA procedure:

3.10.2.1. Standards Preparation:

The protocol provided by the supplier was used. The lyophilized Human IL-10 standard was reconstituted by adding 0.2ml of 1X Assay Diluent A to make the 150ng/ml standard stock solution. The standard was mixed to ensure complete reconstitution and allowed to sit for 15 minutes with gentle agitation prior to making dilutions. 1000µl of the top standard at 250pg/ml by adding 16.7µl of reconstituted standard stock solution to 983.3µl 1X assay diluent A. Prepared six two-fold serial dilutions of 250pg/ml top standard with 500ml of assay diluents in 6 tubes labeled as 250 pg/ml, 125pg/ml,62.5pg/ml, 31.3pg/ml, 15.6pg/ml, 7.8pg/ml. Calibrator diluents served as the zero standard (B0) (0/pg/ml).[106]

3.10.2.2. Assay Procedure:

The ELISA procedure was performed according to the instructions of the supplier (Biolegend, USA). Briefly, plate was coated by adding 100µl capture antibody solution to each well and incubated for overnight in 4°C. Following incubation, the plate was washed 4 times and blocked by adding 200µl 1X assay diluent to each well, and then the plate was incubated in a shaker at room temperature for 1 hour. Following washing 4 times. 100µl of standard, controls, and samples were added to the appropriate wells. Next, the plate was incubated at room temperature and washed 4 times. Then 100 µl of the diluted detection antibody solution were added to each well. Plates were incubated for 1 hour at room temperature on a micro plate shaker. Then 100 µl of avidin horseradish peroxdase HRP was added to each well and the plates were covered with plate sealer. Plates again incubated on the shaker for 30 minutes, then washed 5 times before the addition of 100 µl of substrate solution. Reactions were terminated by adding 100 µl /well of Stop

solution. Plates were then read immediately at 450 (Versa Max microplate reader, Molecular Devices).

Resulting OD was obtained by average between each two readings. IL-10 concentration of samples was extrapolated from a four parameter logistic standard curve constructed in Graph pad prism six using the mean values obtained for the calibrators.[106]

3.10.3. Spectrophotometric procedure for ALT and AST:

3.10.3.1. Method of Aspartate aminotransferase (AST):

Aspartate aminotransferase(AST or GOT) Catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate,forming oxaloacetate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340nm, means of the malate dehydrogenase(MDH) coupled reaction.[107,108,109]

The Working reagent prepared by poured the contents of the reagent B into the reagent A bottle then mixed gently. The procedure of the test done by pipetting into a cuvette 1ml of working reagent, added 50 µl from sample, mixed and insert the cuvette into the photometer, started the stopwatch, after 1minute recorded initial absorbance and at 1 minute intervals thereafter for 3 minutes, calculated the difference between consecutive absorbance, and the average absorbance difference per minute. Calculated result according to calculate formula the factor was 1746U/L or 29.1µkat/L.[110]

3.10.3.2. Method of Alanine aminotransferase (ALT):

Alanine aminotransferase(ALT or GPT) Catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate,forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340nm, means of the lactate dehydrogenase(LDH) coupled reaction.[111,108,109]

The Working reagent prepared by poured the contents of the reagent B into the reagent A bottle then mixed gently. The procedure of the test done by pipetted into a cuvette 1ml of working reagent, added 50 µl from sample, mixed and insert the cuvette into the photometer, started the stopwatch, after 1minute recorded initial absorbance and at 1 minute intervals thereafter for 3 minutes, calculated the difference between consecutive absorbance, and the average absorbance difference per minute, calculated result according to calculate formula the factor was 1746U/L or 29.1µkat/L.[110]

3.11. Ethical considerations:

The ethical consideration of this study was approved by ethics committee, faculty of graduate studies, Shendi University, Sudan. The participants were informed about the purpose of the research before sample collection, and verbal consent obtained from them. Privacy and confidentiality of participants were ensured.

CHAPTER FOUR

RESULTS

A total of 60 blood sample collected from HBV patients as case group and 30 samples collected as control group from HBV free individuals. Out of the 60 HBV patients, 48 (80 %) were male and 24 (20 %) were female as shown in Figure 4.1.
The age of study population was ranged from 27 to 70 years most of them 36 (60%) were above 40 years old and only 24 (40%) were under 40 years old as shown in Figure 4.2.

Out of the 60 HBV patients, 20(33.3%) were acute HBV patients, 20(33.3%) were chronic HBV patients, 10(16.7%) were HBV patients with liver cirrhosis and 10(16.7%) were HBV carriers as shown in (Table 4.1).
The study showed that there was no significant statistical correlation between the age, sex and clinical stages of HBV infection as shown in (Table 4.2) and (Table 4.3)

The study revealed that there was significant statistical elevation in the level of mean ALT, AST and IL-10 in case group when compared with control group, in which the mean level of ALT in case group was 105.6.u/l in case group and only 27.3u/l in control group, the mean level of AST in case group was 90.2u/l in case group and only 24.3u/l in control group, and the mean level of IL-10 in case group was 18.2ng/ml in case group and only 10.1ng/ml in control group as shown in (Table 4.4).

The study revealed that there was significant statistical difference in the level of mean ALT, AST and IL-10 among the different clinical stages of HBV infection, in which the mean level of ALT was 120.3u/l in acute stage, 137.7u/l in chronic stage, 67.4u/l in liver cirrhosis stage and 52.7u/l in carriers stage. The mean level of AST was 81.9u/l in acute stage, 119.5u/l in chronic stage, 88.3u/l in

liver cirrhosis stage and 56.1u/l in carriers stage. The mean level of IL-10 was 11.7 ng/ml in acute stage, 7.2 ng/ml in chronic stage, 60.3 ng/ml in liver cirrhosis stage and 11.0 ng/ml in carriers stage.as shown in (Table 4.5).

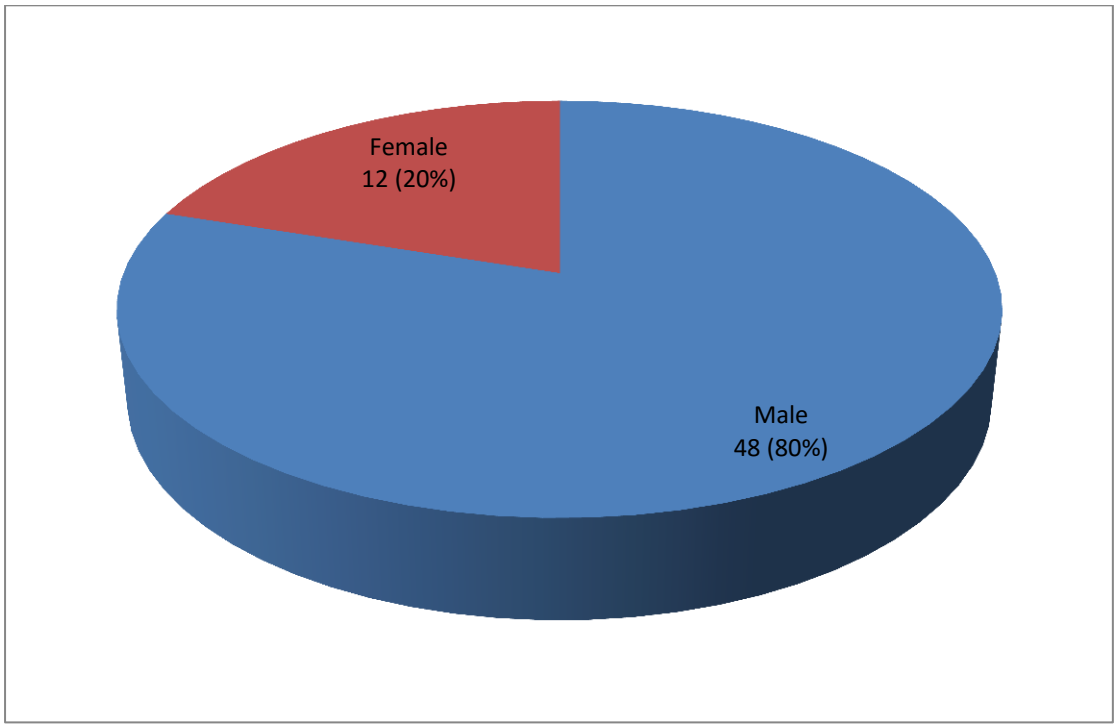

Figure (4.1) distribution of HBV patients cording to gender

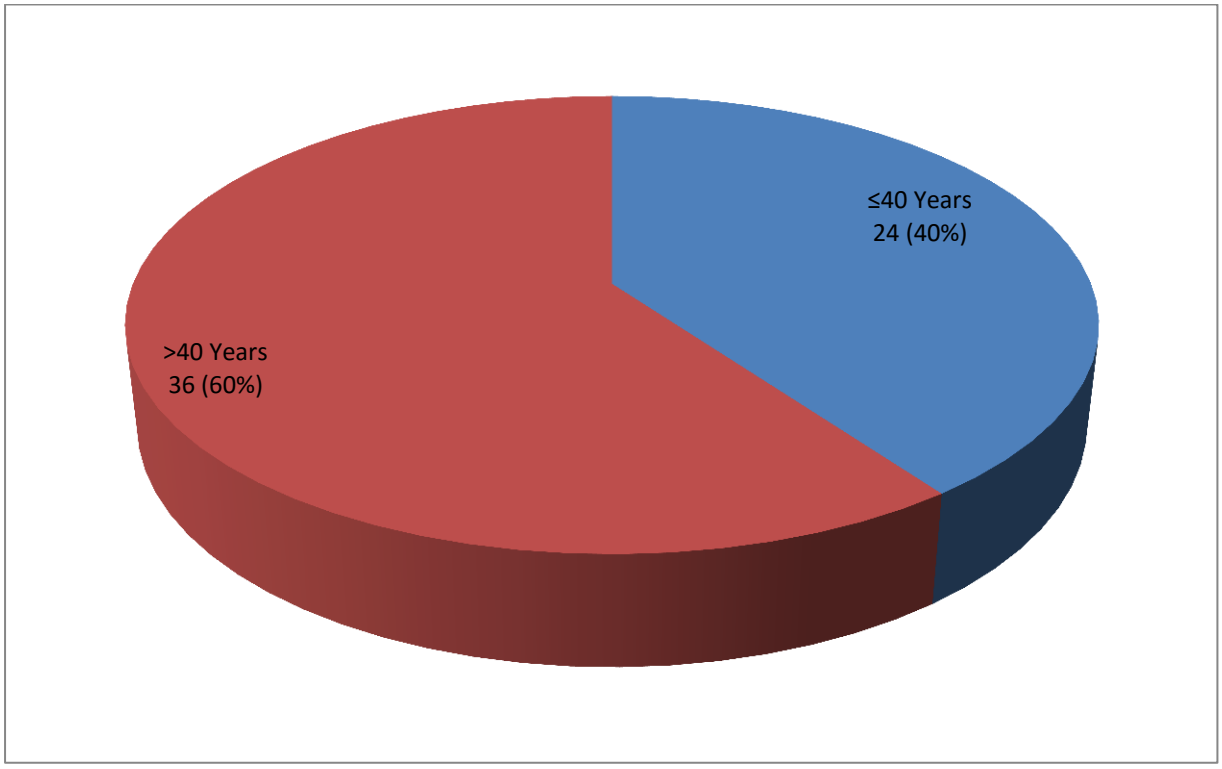

Figure (4.2) distribution of HBV patients cording to age group

Table (4.1). Shows the distribution of study population according to clinical stages group.

Clinical stage	Frequency	Percent
Acute	20	33.3
Carrier	10	16.7
Chronic	20	33.3
Cirrhosis	10	16.7
Total	60	100%

Table (4.2). shows the relationship between the age and clinical stages

Clinical diagnosis	Age		P-value
	≤40 Years	>40 Years	
Acute	6 (30%)	14 (70%)	0.373
Chronic	7 (35%)	13 (65%)	
Carrier	5 (50%)	5 (50%)	
Cirrhosis	6 (60%	4 (40%)	
Total	24 (40.0%)	36 (60.0%)	

Table(4.3) shows the relationship between the gender and clinical stages

Clinical stage of hepatitis B	**Gender of case**		**P value**
	Male	**Female**	
Acute	**16**	**4**	**0.329**
Carrier	**10**	**0**	
Chronic	**15**	**5**	
Cirrhosis	**7**	**3**	
Total	**48**	**12**	

Table (4.4) shows the mean comparison between case and control in serum level of ALT, AST and IL10.

Parameters	**Case (Mean±SD)**	**Control (Mean±SD)**	**P-value**
ALT	**105.6±13.78**	**27.27±12.87**	**0.000**
AST	**90.15±78.08**	**24.33±12.13**	**0.000**
IL10	**18.15±10.28**	**10.10±9.12**	**0.042**

Table (4.5)shows the mean comparison among different Clinical stages of HBV in serum level of ALT, AST and IL10 .

Parameters	Mean±SD				**P-value**
	Acute	Chronic	Carrier	Cirrhosis	
ALT	120.3±23.63	137.7±28.86**	52.70±16.93**	67.40±22.99**	0.004
AST	81.90±72.81	119.5±94.38**	56.10±55.99**	88.30±53.13Ns	0.023
IL10	11.70±4.35	7.20±1.88Ns	11.00±3.72Ns	60.30±13.26**	0.000

CHAPTER FIVE

5.1. DISCUSSION

This study was conducted to determine the association of IL-10 with different stages of HBV infection. A total of 90 participants were involved, 60 of them were HBV positive with different clinical stages as case group and 30 of them were free from HBV as control group.

The present study showed that IL-10 level in HBV patient groups was significantly increased compared with the control group (18.15ng/ml in case, 10.1ng/ml in control, p=0.04). These finding were agreement with that obtained by Tulek. et al, (2000) who reported that 10 levels were significantly higher in chronic hepatitis cases and asymptomatic carriers than that of others ($P < 0.01$). [112]

The present study also showed that the mean of IL-10 was prominently highest in cirrhotic group (60.3ng/ml), when compared with other HBV clinical stages(acute, chronic and carriers), this difference was statistically significant (p=0.000). This result was in close agreement with that obtained by Rolix, (2014), who found that IL-10 level was highest among cirrhotic than other stages.[102]

In this study the mean of ALT level was higher in case group than control group (105.6u/l in case, 27.27u/l in control), which was statistically significant (p=0.000), this result was in agreement of result obtained by Stancoven, (2005), who found that the mean of ALT level in active HBV infections was higher than HBV negative controls.[113] This result may be attributed to the small sample size of the present study, and may be the patients in last days in acute stage.

In this study the mean of AST level was higher in case group than control group (90.15u/l in case, 24.33u/l in control), , this finding is agrees with that study

obtained by Chai-Jan Chang, et al (2000) who reported the HBsAg-positive group had a higher AST level than the negative group.[114]

The study revealed that the chronic HBV patients showed the highest level of mean serum ALT (137.7u/l) and AST(119.5u/l) when compared with other HBV clinical stages(acute, carrier and cirrhotic), which was statistically significant (p= 0.004 for ALT and p=0.023 for AST). These results were in agreement of that obtained by Jian-Qi. et al, (2014) who found that the highest level of serum ALT and AST was found in chronic HBV.[115]

The study showed no statistical correlation between the gender and clinical stages of HBV infection, this result was different with study done by Fawad et al.,(2011) who reported that male were found to be more frequently infected as compared to the female with positivity ratio of (2.14: 1.0) respectively.[116] This difference may be attributed to the small sample size of the present study.

The study also denoted that no statistical correlation between the age of participants and clinical stages of HBV infection, this finding disagrees with that obtained by Fawad et al., (2011) who reported the Younger age group had significantly high rate of HBV infection as compared to the children's and the older age groups. [116]

5.2. Conclusion

.This study concluded that the IL-10 level high in cirrhosis than other groups (60.30ng/ml in cirrhotic, 10.1ng/ml in control). Have significant marker contribution in disease progression.

The level of ALT, and AST was significantly elevated in case group when compared with control group, mean of IL-10(18.15ng/ml in case, mean of ALT (105.6u/l in case, 27.27u/l in control), and mean of AST(90.15u/l in case, 24.33u/l in control).

2.3.Recommendation

1. Further study with large sample size is needed.
2. Regular monitoring of HBV patients regarding IL-10 level and AST and ALT level are important for those patients

REFERENCES

1. N. J. Dimmock, A. J. Easton and K. N. Leppard. *Introduction to Modern Virology*. 7th edition. UK: Wiley & Sons, West Sussex; 2016:347-363.
2. Kumar V, Abbas A, Fausto N and Mitchell R. Robbins. *basic pathology*. 8th edition. Saunders; 2007:663-666.
3. TESTSURA IS HIKAWA. Immunoregulation of Hepatitis B Virus Infection – Ratonale and Clinical Application . *Nagoya Journal Of Medical Science*. 2012; Aug;(74) 3-4.
4. Mahoney FJ, Kane M. Hepatitis B vaccine. In: Plotkin SA, Orenstein WA,eds**.** *Vaccines*, 3rd edition. Philadelphia: W.B, Saunders Company; 1999:158-182.
5. Viral Hepatitis Prevention Board. Universal HB immunization by 1997: 1998.(Fact Sheet VHPB /1998 /2, (http://hgins.uia.ac.be/esoc /VHPB/vhfs2.html).
6. Hollinger FB, Liang TJ. Hepatitis B Virus. In: Knipe DM et al. *Fields Virology*. 4th edition. Philadelphia: Lippincott Williams & Wilkins; 2001:2971-3036.
7. Francis V. Chisari, Masanori Isogawa, and Stefan F.Wieland. Pathogenesis of Hepatitis B Virus Infection. *PatholBiol (Paris).* 2010; 58(4): 258–266.
8. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med*. 1991; 174:1209–1220.
9. Guidotti LG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol*, 2001; 19: 65–91.
10. Horng-TayTzeng, Hwei-Fang Tsai, Hsiu-Jung Liao, Yi-Jiun Lin, Lieping Chen, Pei-Jer Chen and Ping-Ning Hsu. PD-1 Blockage Reverses Immune Dysfunction and Hepatitis B Viral Persistence in a Mouse Animal Model. *PLoS ONE*. 2012; 7 (6):e39179.

11. Ouyang, W. et al. Regulation and function of the IL10 family of cytokines in inflammation and disease. *Annu. Rev. Immunol. 2011; 29:71*

12. Saraiva, M. and A. The regulation of IL10 producing by immune cell. O'Gara, *Nat. Rev. Immunol*.2010; 10:170 .

13. Vieira, P. et al**.** Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: homology to Epstein-Bae virus open reading frame BCRFI. *Proc. Natl. Acad Sci USA*. 1991; 88:1172.

14. Hsu, D.-H. et al. Expiration of interlukin10 activity by Epstein-Bar virus protein BCRF1. *Science*.1990; 250:830.

15. Windsor, W.T,Syto R, Tsarbopoulos.A, et al**.** Disulfide bond assessments and secondary structure analysis of human and murine interlukin 10.Biochemistry.1993; 32:8807-15.

16. Syto, R. et al. (1998) Biochemistry 37:16943

17. Mathurin, P. *et al.* IL10 receptor and coreceptor expression in quiescent and activated hepatic stellate cell. *Am. J. Physiol. Gastrointest. Liver Physiol*.2002; 282:G981

18. Ledeboer, A.Breve JJ, Wierinckx A et al. Expression and regulation of interleukin 10 and interleukin 10 receptor in rat astroglial and microglial cells. *Eur. J. Neuro sci*. 2002; 16:1175.

19. Kotenko, S.V. *et al*. *Identification and functional characterization of asecond chain of interleukin 10 receptor complex. EMBO J*.1997; 16:5894.

20. Lutfalla, G. et al**.** An4ew member of the cytokine receptor gene family maps on chromosome 21 at less than 35 kb from INFAR. *Genomics*.1993 ; 276:2725

21. Kotenko, S.V., Saccani,S., Izotova,L.S., Mirochintchenko, O.V., and Pestka, S. Human cytomegalovirus harbors its own unique IL-10 homolog(cmv IL10).Proc. Natl. Acad. Sci. USA. 2000;1695-1700.

22. Hor, S.,Pirzer, H., Dumoutier, l. Bauer,F. et al. The T.cell lymphokine interleukin -26 targets epithelial cell through the interleukin 20 receptor 1 and interleukin 10 receptor 2 chain. *J. Biol. Chem*.2004; 279:33343.

23. WHO. HBV fact sheet. 2015.(www.who.int) http://www.who.int/mediacentre/factsheets/fs204/en/.Access19/1/2016.

24. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K and Aboyans.V**.** Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012; 380:2095-2128.

25. Timothy M.Blocck ,ph.D.,HaitaoGuo , ph.D. , and Ju-Tao Guo,M.D. Molecular Virology of Hepatitis B Virus For Clinicians .*National Institutes Of Health* . 2007 November ;11(4):685-706.

26. T . Jake Liang. Hepatitis B: The Virus And Disease. *National Institutes Of Health*. 2009May; 49(5suppI):s13-s21. doi:10.1002/hep.22881.

27. Hajodelius ,Nicholas M, Gough and et al. Structure Of The Hepatitis B Virus Genome . *Journal Of Virology*. Aug.1983; p.337-343.

28. NeseI NAN ,Fehmi TABAK. Hepatitis B Virus: Biology And Life Cycle. *Viral Hepatitis Journal* .2015 ; 21(1) :1-7.

29. JuergenBeck, Michael Nassal. Hepatitis B Virus Replication. *World Journal Of Gastroenterol* .2007 January 7 ; 13(1) : 48-64.

30. Kay, A. and Zoulim, F. Hepatitis B virus genetic variability and evolution. *Virus research*, 2007; 127(2), pp.164-176.

31. Buti, M, Rodriguez-Frias, F, Jardi, R. and Esteban, R. Hepatitis B virus genome variability and disease progression: the impact of pre-core mutants and HBV genotypes. *Journal of clinical virology*. 2005; 34, pp.S79-S82.

32. Beck, J, Nassal. M. Hepatitis B virus replication. *World journal of gastroenterology* (WJG). 2007; 13(1), p.48.

33. Li. W, Miao. X, Qi. Z, Zeng.W, Lia. J, and Liang. Z. Hepatitis B virus X protein up regulates HSP90alpha expression via activation of c-Myc in human hepatocarcinoma cell line, HepG2. *Virology journal*. 2010; 7(1), p.45.

34. Kramvis. A, Kew. M. and François. G. Hepatitis B virus genotypes. *Vaccine*.2005;23(19), pp.2409-2423.

35. Magnius. L, and Norder. H. Subtypes, genotypes and molecular epidemiology of the hepatitis B virus as reflected by sequence variability of the S-gene. *Intervirology*. 1995; *38*(1-2), pp.24-34.

36. Araujo, N.M, Hepatitis B virus intergenotypic recombinants worldwide: an overview. *Infection, Genetics and Evolution*. 2015; 36, pp.500-510.

37. Norder, H, Couroucé, A.M. and Magnius, L.O. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology*. 1994; 198(2), pp.489-503

38. Shibayama, T., Masuda, G., Ajisawa, A., Hiruma, K., Tsuda, F., Nishizawa, T., Takahashi, M. and Okamoto, H. Characterization of seven genotypes (A to E, G and H) of hepatitis B virus recovered from Japanese patients infected with human immunodeficiency virus type 1. *Journal of medical virology*. 2005; 76(1), pp.24-32.

39. Schaefer, S**.** Hepatitis B virus taxonomy and hepatitis B virus genotypes. *World journal of gastroenterology(WJG).* 2007; 13(1), p.14.

40. Keith R. Jerome. Lennettes. *Laboratory Diagnosis Of Viral Infection*. 4th edition. CRC Press; 2010: 325-400.

41. Hatim My Mudawi. Epidemiology Of Viral Hepatitis in Sudan. *Clinical and Experimental Gastroenterology* 2009 :1 9-13.

42. Miriam J Alter. Epidemiology Of Hepatitis B In Europe And Worldwide. *Journal Of Hepatology*. February 2003; Doi : 10.1016/s0168-8278 (03) 00141-7.

43. Francis.V, Chsari, Masanori Icogawa, and Stefan F.Wieland. Pathogenesis Of Hepatitis B Virus Infection. *National Institutes Of Health*. 2010 August ; 58 (4) : 258-266 . doi : 10.1016/j.patpio . 2009 . 11.001.

44. Francis. J, Mhoney. Update On Diagnosis , Management, And Prevention Of Hepatitis B Virus Infection. *Clinical Microbiology Reviews.* Apr 1999; p 351-366

45. Yan, H., Zhong, G., Xu, G., He, W., Jing, Z., Gao, Z., Huang, Y., Qi, Y., Peng, B., Wang, H. and Fu, L. *Sodium taurocholateco transporting polypeptide is a functional receptor for human hepatitis B and D virus.* Elife. 2012;1.

46. Tong, S., Li, J. and Wands, J.R. Carboxypeptidase D is an avian hepatitis B virus receptor. *Journal of virology*. 1999;73(10), pp.8696-8702.

47. Glebe, D. and Urban, S. Viral and cellular determinants involved in hepadnaviral entry. *World journal of gastroenterology: WJG*.2007; 13(1), p.22.

48. Coffin, C.S., Mulrooney-Cousins, P.M., van Marle, G., Roberts, J.P., Michalak, T.I. and Terrault, N.A. Hepatitis B virus quasispecies in hepatic and extrahepatic viral reservoirs in liver transplant recipients on prophylactic therapy. *Liver Transplantation*.2011;17(8), pp.955-962.

49. Iannacone, M., Sitia, G., Ruggeri, Z.M. and Guidotti, L.G**.** HBV pathogenesis in animal models: recent advances on the role of platelets. *Journal of hepatology*. 2007; 46(4), pp.719-726

50. Iannacone, M., Sitia, G., Isogawa, M., Marchese, P., Castro, M.G., Lowenstein, P.R., Chisari, F.V., Ruggeri, Z.M. and Guidotti, L.G. Platelets mediate cytotoxic T lymphocyte–induced liver damage. *Nature medicine* 2005;11(11), p.1167.

51. Terrault N, Roche B, Samuel. D. Management of the hepatitis B virus in the liver transplantation setting: a European and an American perspective. *Liver Transplant.* July2005; 11(7): 716 – 32. doi: 10.1002 / lt. 20492. PMID 15973718.

52. Jake Liang. Hepatitis B : The Virus And Disease. *National Institutes Of Health*. 2009 May; 49(5suppI): s13-s21. doi:10.1002/hep.22881.

53. Jump up^ El-Serag H.B, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology*. June 2007; 132 (7):255776. doi:10. 1053/ j. gastro.04.061. PMID 17570226.

54. Jump up^ El-Serag H.B. "Hepatocellular carcinoma. *New England Journal of Medicine.* 22 September 2011; 365 (12):111827. doi:10.1056/NEJMra1001683. PMID 21992124.

55. Jump up^ Gan SI, Devlin SM, Scott-Douglas NW, Burak KW. Lamivudine for the treatment of membranous glomerulopathy secondary to chronic hepatitis B infection. *Canadian journal of gastroenterology = Journal canadien de gastroenterologie*. October 2005; 19 (10): 625–9. PMID 16247526.

56. Jump up^ Dienstag JL. "Hepatitis B as an immune complex disease Seminars in Liver Disease. February1981.1 (1) :4557. doi:10.1055/s20081063929. PMID 6126007.

57. Jump up^ Trepo C, Guillevin. "Polyarteritisnodosa and extrahepatic manifestations of HBV infection: the case against autoimmune intervention in pathogenesis". *Journal of Autoimmunity.* May 2001; 16 (3): 269–74. doi:10.1006/jaut. 2000.0502. PMID 11334492.

58. Jump up^ Alpert E, Isselbacher KJ, Schur PH. "The pathogenesis of arthritis associated with viral hepatitis. Complement-component studies". *The New England Journal of Medicine.* July 1971; 285 (4):185–9. doi: 10.1056 / NEJM 197107222850401. PMID 4996611.

59. Jump up^ to:a b c Liang TJ. "Hepatitis B: the virus and disease". *Hepatology*. May 2009; 49 (5Suppl): S13–21. doi:10.1002/hep.22881. PMC 2809016. PMID 19399811.

60. Jump up^ Gocke DJ, Hsu K, Morgan C, Bombardieri S, Lockshin M, Christian CL, "Association between polyarteritis and Australia antigen". *Lancet*. December 1970; 2 (7684):1149–53. doi:10.1016/S0140-6736(70) 90339 -9. PMID 4098431.

61. Jump up^ Lai KN, Li PK, Lui SF, Au TC, Tam JS, Tong KL, Lai FM**.** Membranous nephropathy related to hepatitis B virus in adults. *The New England Journal of Medicine*. May1991.324 (21): 145763. doi:10.1056/ NEJM 199105233242103. PMID 2023605.

62. Jump up^ Takekoshi Y, Tanaka M, Shida N, Satake Y, Saheki. Y, Matsumoto. S. "Strong association between membranous nephropathy and hepatitis-B surface antigen aemiain Japanese children". *Lancet***.** November 1978; 2 (8099):10658. doi:10.1016 / S01406736(78)91801-9. PMID 82085

63. Kumar V, Abbas AK, Aster JC, Editors, Robbins &Contran. *Pathologic Basis of Diseases*. 9th ed. Philadelphia: Elsevier; 2014

64. Salem R, Lewandowski RJ. Chemoembolization and radioembolization for hepatocellular carcinoma. *Clin Gastroenterol Hepatol*. 2013;11:604

65. Carrilho FJ, Kikuchi L, Branco F, Goncalves CS, Mattos AA. Brazilian HCC Study Group. Clinical and epidemiological aspects of hepatocellular carcinoma in Brazil. *Clinics (Sao Paulo*). 2010;65:1295-90.

66. Huguet C, Stipa F, Gavelli A**.** Primary hepatocelular cancer: western experience. In: Blumgart LH, editor. Surgery of the Liver and Biliary Tract. *New York: Churchill Living stone*; 1996; Pp. 1365-1369.

67. Ebara M, Hatano R, Fukuda H, Yoshikawa M, Sugiura N, Saisho H. Natural course of small hepatocellular carcinoma with underlying cirrhosis. *Hepatogastroenterology* 1998;45: Suppl 3:1214-20.

68. Bruix J, Reig M, Sherman M**.** Evidence-Based Diagnosis, Staging, and Treatment of Patients With Hepatocellular Carcinoma. *Gastroenterology*. 2016;150:835-53.

69. Hsu HC, Sep JC, Lin YH, et al. Prognostic histologic features of resected small hepatocellular carcinoma (HCC) in Taiwan. A comparison with resected large HCC. *Cancer* 1985;56:672-80.

70. Hong Y, Wu LP, Ye F, Zhou YM. Adjuvant Intrahepatic Injection Iodine-131 Lipiodol Improves Prognosis of Patients with Hepatocellular Carcinoma After Resection: a Meta-Analysis. *Indian J Surg*. 2015;77(Suppl 3):1228-32.

71. Pratt DS, and Kaplan MM**.** Evaluation of abnormal liverenzyme results in asymptomatic patients. *N Engl J Med. 2000;* 342:1266-1271

72. Nalpas B, Vassault A, Le Guillou A, Lesgourgues B, Ferry N, Lacour B and Berthelot P. Serum activity of mitochondrial aspartate aminotransferase: a sensitive marker of alcoholism with or without alcoholic hepatitis. *Hepatology*.1984; 4:893-896.

73. Salaspuro. M. Use of enzymes for the diagnosis of alcohol-related organ damage. *Enzyme*.1987; 37:87-107.

74. Sheth SG, Flamm SL, Gordon FD and ChopraS, (1998): AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. *Am J Gastroenterol*.1998; 93:44-48

75. Diehl AM, Potter J, Boitnott J, Van Duyn MA, Herlong HF and Mezey E**.** Relationship between pyridoxal 5'-phosphate deficiency and aminotransferase levels in alcoholic hepatitis. *Gastroenterology*.1984; 86:632-636

76. Interpretation of Hepatitis B Serologic Test Results. 2017;8.5.7.https://www.cdc.gov/hepatitis/hbv/pdfs/serologicchartv

77. Pommervillej. Alcomos. *Fundamentals of microbiology*.7thed London : Oxford University Press. 2004.

78. Margaret Supran , Elizabeth H Boxall , J Craske ,Rjc Hart Elise M Vander Velde, Ps Gardner. Enzyme-Linked Immunosorbent Assay For The Detection Of Hepatitis Be Antigen And Antibody : Report Of a Field Trial . *J CLIN Pathol*.198 ; 36 :581-585.

79. Shuichi Kaneko .Roger H et al. Detection Of Serum Hepatitis B Virus DNA In Patients With Chronic Hepatitis Using The Polymerase Chain Reaction Assay. *Medical Sciences*. January 1989; vol .86 , pp . 312-316 ,

80. P .J . M .Rijntjes ,Th . J .M, Van Ditzhuijs En, et al. Hepatitis B Virus DNA Detected In Formalin-Fixed Liver Specimens And Its Relation To Serologic Marker and Histopathologic Features In Chronic Liver Disease . *American Journal Of Pathology*. 1985; 120 : 411-418.

81. Bell, S.J. and Nguyen, T. The management of hepatitis B. *Australian Prescriber*.2009; 32(4), pp.99-104

82. Dienstag, J.L**.** Hepatitis B virus infection. *New England Journal of Medici.* 2008; 359(14), pp.1486-1500.

83. Kerkar, N., (2005). Hepatitis B in children: complexities in management. *Pediatric transplantation*.2005; 9(5), pp.685-691.Jun-

84. Taylor, J.M. Hepatitis delta virus. *Virology*.2006; 344(1), pp.71-76.

85. Oliveri, F., Brunetto, M.R., Actis, G.C. and Bonino, F. (1991).Pathobiology of chronic hepatitis virus infection and hepatocellular

86. Schillie, S.F., Murphy, T.V., Sawyer, M., Ly, K., Hughes, E., Jiles, R., de Perio, M.A., Reilly, M., Byrd, K. and Ward, J.W. CDC guidance for evaluating health-care personnel for hepatitis B virus protection and for administering post exposure management.2013.

87. COMMITTEE ON INFECTIOUS DISEASES. Elimination of Perinatal Hepatitis B: Providing the First Vaccine Dose Within 24 Hours of Birth. *Pediatrics*.2017; p.e20171870.

88. Lee, C.F. hepatitis B immunization for new barns of hepatitis B surface antigen positive mothers : acochrane hepato biliary group systematic review and meta-analysis. *Value in Health*2006; 9(3), p.A43Pg11.

89. Wong, F., Pa, R., Van Schalkwyk, J. and Yoshida, E.M. Hepatitis B in pregnancy: a concise review of neonatal vertical transmission and antiviral prophylaxis. *Annals of Hepatology*: *Official Journal of the Mexican Association of Hepatology*.2014; 13(2).

90. Hyun, M.H., Lee, Y.S., Kim, J.H., Je, J.H., Yoo, Y.J., Yeon, J.E. and Byun, K.S. Systematic review with meta-analysis: the efficacy and safety of tenofovir to prevent mother-to-child transmission of hepatitis B virus. *Alimentary pharmacology & therapeutics*.2017;45(12), pp.1493-1505.

91. Eke, A.C., Eke, U.A. and Uchenna, E., 2010. Hepatitis B immunoglobulin during pregnancy for the prevention of mother to child transmission of hepatitis B virus. *Protocol Cochrane collaboration. 2010.*

92. Sangkomkamhang, U.S., Lumbiganon, P. and Laopaiboon, M. Hepatitis B vaccination during pregnancy for preventing infant infection. *Cochrane Database Syst Rev*.2011; 3.

93. Chen, W. and Gluud, C. Vaccines for preventing hepatitis B in health-care workers. *The Cochrane Library*.2005;(2):27-35

94. Lutgens, S.P., Nelissen, E.C., van Loo, I.H., Koek, G.H., Derhaag, J.G. and Dunselman, G.A. To do or not to do: IVF and ICSI in chronic hepatitis B virus carriers. *Human reproduction*. 2009; 24(11), pp.2676-2678

95. LeFevre, M.L. Screening for hepatitis B virus infection in nonpregnant adolescents and adults: US Preventive Services Task Force recommendation statement. *Annals of internal medicine*.2014;161(1), pp.58-66.

96. Christoph Seeger, Williams. Mason. Molecular Biology Of Hepatitis B Virus Infection .*Virology*.2015 . 479-480 .672-686.

97. Souza R , MRCP, Faster A. Diagnosis And Treatment Of Chronic Hepatitis B. *Journal Research Society*. 2004 ;77(7) , 318-321.

98. Jun-Ming Zhang, Msc, MD and Jianxiong An, Msc, MD. Cytokines,Inflammation and Pain. *National Institutes Of Health* . 2007; 45(2) : 27-37 E.

99. Shanker Subramanian Her and Genhong Chengo. Role Of Interleukin-10 Transcriptional and Autoimmune Disease. *Crit Rev Immunol*.2012; 32 (1):23-63.

100. Wang K, Wuz B, Yu YN, et al. Plasma Interleukin-10 : Alikely Predictive Marker For Hepatitis B Virus – Related Acute-on-Chronic Liver Failure. *Hepat Mon Journal* .2014 Julay;14 ;14(7):e19370.

101. OzgularM, Akbulut HH, AkbulutA**.** Evaluation Of Interleukin-10 Levels in Patients Diagnosed With Chronic Hepatitis. *West Indian Medical Journal.* 2015; Mar.64(2):71-5.

102. RoliSaxen ,Yogesh, Kaumar,Chawla, InduVerma, Jyotdeep, Kaur. Association Of Interleukin-10 With Hepatitis B Virus (HBV) Mediated Disease Progression in Indian Population. *Indian J Med* . 201;139 (5) :737-745.

103. Fazal Karim, Salimur Rahman, Mobin Khan, et al. Serum Interleukin-10 Level in Patients With Chronic Hepatitis B Infection. *Hungarian Medical Journal* .June 2007; 1(2):241-250.

104. Bozkaya H, Bozdayi M, Türkyilmaz R, Sarioglu M, Cetinkaya H, Cinar K, Kose k,Yurdaydin C, Uzunalimoglu.O. Circulating IL-2, IL-10 and TNF-alpha in chronic hepatitis B: their relations to HBeAg status and the activity of liver disease. *Hepatogastroenterology* 2000; 47 :1675-9.

105. Cheesbrough M**.** District laboratory practice in tropical countries, Part2. Cambridge University Press, Cambridge: UK. 2006: 297-298.

106. 0Product datasheet. Human IL-10 ELISA maxTM deluxe set.www.biolegend.com/media_assets/pro_detail/datasheets/430604. 15.6.2017.

107. IFCC primary reference procedure for measurement of catalytic activity concentrations of enzymes at 37c̊. part4.Reference procedure for the measurement of catalytic concentration of aspartate aminotransferase. *ClinChem Lab Med* 2002;40:725-733.

108. IFCC primary reference procedure for measurement of catalytic concentrations of enzymes: corrigendum, notes and useful advice. *ClinChem Lab Med* 2010;48:615-621.

109. Gella FJ, Olivella T, Cruz pastor M, Arenas J, Moreno R, Durban R, and Gomez JA. A simple procedure for routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. *ClinChimActa* 1985;153-247-241-247.

110. Bio systems**.** (Spain).

111. IFCC primary reference procedure for measurement of catalytic concentrations of enzymes at 37c̊**.** part4,Reference procedure for the measurement of catalytic concentration of alanie aminotransferase. *Clin Chem Lab Med* 2002;40:718-724.

112. Tulek N, Saglam SK, Saglam M, Turkyilmaz R, Yildiz M**.** Soluble interleukin-2 receptor and interleukin-10 levels in patients with chronic hepatitis B infection. *Hepatogastroenterology* 2000; 47 : 828-31.

113. Stancoven K, Hwang L.-Y, Troisi C.L. Significance of Anti-HBc in the Absence of Other Hepatitis B Markers in a College-Aged Population, *Intervirology* 2005;48:273–278 (DOI: 10.1159 / 000085094).

114. Chai-Jan Chang, MD,Ying-Chin KO,PhD, and Hong-Wen LIU, MD. Digestive Disease and Sciences. October 2000; Vol.45, No.10 pp.1949-1952.

115. Jian-Qi lian; Xiano-fei Yang; Rong-Rong Zhao,Yan-Yan Zhao,Yuli,Ye Zhang,Chang-Xing Huang, Expression profiles of circulating

cytokines,chemokines and immune cell in pts with heptitis B virus infection. *Hepat Mon.* 2014; 14(6):e188892.

116. Fawad Khan, Sulaiman Shams, Ihteshamud, Muhmmad Israr,Hayat Khan, Muhammad Tahir Sarwar, and Muhammad Ilyas. Hepatitis B virus infection among different sex and age groups in Pakistani Punjab, *Virol J.* 2011; 8: 225, PMID: 21569532.

APPENDIX I

Association of Interleukin-10 with different Clinical Stages of Hepatitis B Virus Infection in IbnSina Hospital-Khartoum State; 2018.

Questionnaire

Lab No. …….

Name: ……

Gender: Male: { }

Female: { }

Age: ……..

Patient: case ( ) control ( )

Clinical diagnosis:

Carrier : { }

Acute with Symptoms: { }

Chronic : { }

Liver cirrhosis : { }

Laboratory finding:

ALT level: …….

AST level: …….

IL 10 level: ……..

APPENDIX II

ELISA washer apparatus

APPENDIX III

ELISA reader apparatus

APPENDIX IV

Bio Systems BTS-310