Sudan University of Science and Technology

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Association between Iron Profile and Hepatitis B Viral Load in Sudanese Patients

العلاقه بين مقاييس الحديد والنسخ الفيروسي لإلتهاب الكبد الوبائي(ب) في المرضى السودانين

A thesis submitted in partial fulfillment of the requirement for the degree of M.Sc. In Haematology and immunohematology

By

Alaa Hanafi Mekki Elkhalifa

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Supervisor:

Dr. Salma Elmalieh Abdalla

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الايه

أَعُوذُ بِاللهِ مِنَ الشَّيْطَانِ الرَّجِيم

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

صدق الله العظيم

[طه: 114]

Dedication

To my great parents who provide invaluable support for me throughout of my life.

To my brothers, cousin, special friends and colleagues who represent strong support and encouragement for me

I dedicate this work

Acknowledgment

All thanks go to Allah who I solely depend on for guidance in my life

I am grateful for my supervisor Dr.Salma Elmalieh for her patience guidance and support throughout the study Grateful thanks are extended to my friend and colleague Dr.Sarah Abdel Mahmoud for her true support. Sincere thanks are also extended to Elreyada modern laboratory staff for their help in providing samples, equipment's and space to carry out this study.

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ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CHB	Chronic hepatitis B
CLD	Chronic liver disease
DNA	Deoxyribonucleic acid
FeIII	Ferric
FeII	Ferrous
HBV	Hepatitis B virus
HCC	Hepatocelluler carcinoma
HBsAg	Hepatitis B surface antigen
HBeAg	Hepatitis B enveloped antigen
HBc	Hepatitis B core
ISGs	Interferon incused gens
mRNA	Messenger RNA
RNA	Ribonucleic acid

SI	Serum iron
SPSS	Statistical package for social science
SD	Standard deviation
TIBC	Total iron binding capacity
TS	Transferrin saturation
TFR2	Transferrin receptor 2
P	Probability
PCR	Polymerase chain reaction

Abstract

Hepatitis B Virus infection is a major global public health problem , Chronic hepatitis B virus (HBV) infection affects 240 million people worldwide and can lead to chronic liver diseases including chronic hepatitis B (CHB), cirrhosis, and hepatocellular carcinoma (HCC).This is case control study carried out in Khartoum state in Elriyada medical laboratory aimed to associate the iron profile with chronic hepatitis B viral load , in the period from April to June 2019.Sixty samples were collected from patients with HBV infection and sixty samples were collected from healthy individuals**.** Serum samples were separated from participants for estimation of iron, ferritin , transferrin ,TIBC , ALT and viral load , (DIRUI CST 240) automated chemistry analyzer used for iron ,TIBC, transferrin and ALT , while ferritin was estimated using(TOSOH AIA1800) ,results of viral load was collected from data recorded from Real Time PCR, data was analyzed by SPSS version 23. the mean of serum iron was significant lower in cases group (86.3±36) when compared with control group(129 ±49) the mean of TIBC were significant lower in study group(212.6±56) when compared with control group(374±46),in addition ;the mean of ferritin is also significantly high in cases group(241.6±161) when compared with controls group(86.6±13),no significant difference in transferrin between case and control. When comparing the iron profile in case group between different viral load found that significantly decreased in mean of serum iron in patients with high viral load(82±26) when compared with low(87 ±43)and very low (98±23) also there is significant decreased in mean of TIBC in patient with high viral load(193±35) when compared with low(205±45) and very low (223±65) .ferritin level was increased in high viral load (212±54)when comparing with low(198±54) and very low(161±61) viral load ranges. Also significant negative correlation was found between ALT and viral load level (-.515), Iron metabolism disorders can occur in patients with HBV infection. The serum markers of iron metabolism disorders vary in different level of HBV-activity levels.

المستخلص

تعد عدوى فيروس التهاب الكبد B من المشكلات الرئيسية في مجال الصحة العامة ، حيث تصيب عدوى فيرو)س التهاب الكبد B المزمن 240 مليون شخص في جميع أنحاء العالم ويمكن أن تؤدي إلى أمراض الكبد المزمنة بما في ذلك التهاب الكبد المزمن B (CHB) وتليف الكبد وسرطان الكبد (HCC). هذه دراسه الحالات والشواهد هدفت لتحديد العلاقه بين مقاييس الحديد والنسخ الفيروسي لإلتهاب الكبد الوبائي(ب) في المرضى السودانين أجريت في ولاية الخرطوم في مختبر الريادة الطبي ، في الفترة من ابريل إلى يونيو 2019. تم جمع عينات من المرضى المصابين بعدوى HBV وتمت مطابقة ستون عينه من أفراد أصحاء. تم فصل عينات من المصل من المشاركون لتقدير الحديد ، ferritin ، transferrin ، TIBC، ALT واالنسخ الفيروسي. تم استخدام محلل الكيمياء الآلي ((DIRUI CST 240 للحديد ، TIBC ، الترانسفيرين و ALT ، تم تقدير الفيريتين باستخدام (TOSOH AIA1800 تم جمع نتائج النسخ الفيروسي من البيانات المسجله التي اخذت جهاز real time PCR ، وقد تم تحليل البيانات عن طريق SPSS الإصدار 23. متوسط مصل الحديد كان أقل بشكل ملحوظ في مجموعة الحالات(36±86.3)عند مقارنتها مع مجموعة التحكم(49± 129),متوسط TIBC أيضًا أقل في مجموعة الحالات(56±212.6) عند مقارنتها مع مجموعة التحك(46±374) ، متوسط ferritinزاد معنويا" في مجموعة الحالات(161±241.6) عند مقارنتها بمجموعه الضوابط (13±86.6) ، وليس هناك فرق كبير في قيمه transferrin بين المجموعتين. عند مقارنه مقاييس الحديد في مجموعه الحالات بناءا" على نتائج النسخ الفيروسي وجد ان متوسط الحديد المصلي انخفض معنويا عند النسخ الفيروسي المرتفع(26±82) عند مقارنته مع المنخفض(43± 87) والمنخفض جدا"(23±98)و TIBC في المرضى الذين يعانون من النسخ الفيروسي المرتفع(35±193)عند مقارنتهم بـ المنخفضة(45±205)والمنخفضة جدا(65±223) وزادت قيمة الفيريتين في الحمل الفيروسي المرتفع(54±212) عند مقارنتة مع المنخفض(54±198) والمنخفض جدا(61±161). تم العثور على ارتباط سلبي بين ALT ومستوى الحمل الفيروسي (515.-) . تحدث اضطرابات التمثيل الغذائي للحديد في المرضى الذين يعانون من عدوى HBV. علامات مصل اضطرابات التمثيل الغذائي للحديد تختلف في مستوى مختلف من مستويات النسخ الفيروسي.

CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

1.1Introduction:

Hepatitis B Virus infection is a major global public health problem (Kennedy *et al*, 2010) Chronic hepatitis B virus (HBV) infection affects 240 million people worldwide and can lead to chronic liver diseases including chronic hepatitis B (CHB), cirrhosis, and hepatocellular carcinoma (HCC). About 788000 patients die of HBV-related liver diseases each year (Stanaway *et al.*, 2016). According to (Mueller *et al.*, 2015), Prevalence of hepatitis B infection is highest in sub-Saharan Africa and East Asia. Most people in these regions become infected during childhood and between 5–10% of the adult population are chronically infected. In managing chronic HBV infection, the objective is to detect liver injury early and then stop the progression of liver disease through effective treatment.

Chronic liver disease frequently related to with hematological abnormalities. Anemia of diverse etiology occurs in about 75% of patients of chronic liver disease (Gonzalez-Casas *et al.*, 2009). Maintenance of iron metabolism within the physiological range is essential to human health. The liver synthesizes several proteins that are required for iron metabolism in addition to iron storage. Besides its involvement in iron storage, the liver also produces transferrin and hepcidin, an iron carrier protein in plasma and a hormone regulating iron metabolism, respectively. Another aspect of the relationship between iron and the liver is that this organ is one of the main targets in hemochromatosis (Batts, 2007).

Serum iron (SI), total iron binding capacity (TIBC) and ferritin levels are the principal tests used in the evaluation of iron burden. Another frequently used parameter, transferrin saturation (TS), is calculated by dividing SI level by TIBC, and it shows the percent saturation of transferring.

Chronic liver injury and histological alteration impact iron metabolism and altered iron metabolism may aggravate liver injury (Hino *et al*, 2013; Zou and Sun, 2017). Identification of iron deficiency in these patients is especially important, because it is an easily correctable cause of anemia.

1.2 Rationale

Sudan is classified among the countries with high hepatitis B virus seroprevalence. Hepatitis B virus was the commonest cause of chronic liver disease and hepatocellular carcinoma and was the second commonest cause of acute liver failure in the Sudan (Mudawi 2008). The aim of this study to identify the changes in serum iron components in patients affected with HBV and identify correlations between changes in iron metabolism and HBV activity.

Iron important for progression of liver fibrosis in viral hepatitis, and serum iron parameters, especially ferritin level reflect hepatic iron accumulation (Metwally *et al* 2004). Anemia is very frequent in cirrhotic patients for many different reasons including iron deficiency (Ozatli, *et al* 2000). Identification of iron deficiency in these patients is especially important, because it is an easily correctable cause of anemia.

Total iron binding capacity (TIBC) level changed in hepatic disorders as transferrin is produced in the liver (Jurczyk, *et al*, 2001; Wallach *et al*, and 2007). Ferritin is increased in many patients with acute and chronic liver diseases (CLDs) (Jurczyk, *et al*, 2001; Sikorska et *al*, and 2003). Therefore, serum iron parameters may not truly reflect iron homeostasis in hepatic disorders. It has been proposed that serum iron parameters were unreliable in CLD, and that systemic iron overload should be confirmed histologically in these patients (Di Bisceglie *et al*, 1992).

In managing chronic HBV infection, the objective is to detect liver injury early and then stop the progression of liver disease through effective treatment. To achieve this goal, a clear understanding of various factors involved in HBV pathogenesis is required.

1.3 Objectives

1.3.1 General objective

To Assess iron profile in Sudanese patients with hepatitis B virus infection, Khartoum –Sudan.

1.3.2 Specific objectives:

To measure iron parameters (serum iron, TIBC, transferring and ferritin) levels in patient with HBV.

To compare the level of serum iron, TIBC, transferrin, and ferritin between case and controls.

To correlate the level of serum iron, TIBC, transferring and ferritin between different levels of viral load activity results.

To correlate ALT level and HBV viral load.

CHAPTER II

LITERATURE REVIEW

CHAPTER II

LITERATURE REVIEW

2.1. Classification and structures of Hepatitis B virus

Hepatitis B virus (HBV) is a member of the Hepadnaviridae family. The virion is a double-shelled particle, 40 to 42 nm in diameter. It covered by lipoprotein (envelope) which contains three associated envelope glycoproteins, which known as the surface antigens. The envelope covers the viral nucleocapsid, also called the viral core. The nucleocapsid contains the viral genome, a relaxed-circular, partially duplex DNA of 3.2 kb, and a polymerase enzyme that is responsible for the synthesis of viral DNA in infected cells (Ganem, 1991). In addition to virion, HBV-infected cells produce two distinct subviral lipoprotein particles: 20-nm spheres and filamentous forms of similar diameter. These HBsAg particles contain only envelope glycoproteins and host-derived lipids and typically outnumber the virion by 1000:1 to 10,000:1 (Ganem and Alfred, 2004).

2.1.1HBV Genomic structure and Proteins

The HBV genome has four long open reading frames: The pre S-S region, the pre C-C region, the P coding region and the X coding region , The pre S-S region of the genome encodes the three viral surface antigens(HBsAg) , The pre C–C (pre core–core) region encodes hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg), The Viral polymerase is encoded by the P coding region(Bruss and Ganem, 1991) .The viral X protein (HB x) is encoded by the X open reading frame. HBx modulates host cell signal transduction. It can directly and indirectly affect the expression of the host cell genes and viral genes. HB x activity is a necessity for the replication and spread of the virus (Ganem and Schneider, 2001).

2.2 HBV Genotypes and Distribution

There are eight viral genotypes of HBV in existence confirmed by DNA sequencing of many isolates of the virus. Each genotype has characteristic geographic distribution. These genotypes are A, B, C, D, E, F, G and H (Calvin and Jin, 2005). These eight known HBV genotypes which have distinct geographical distributions, genotype A and E prevail in Africa. While genotype A can be found in sub Saharan Africa and elsewhere in the world, (Kramvis *et al*., 2005).

2.3. HBV Replication cycle

HBV replicate through pre genomic RNA intermediate reverse transcription Infectious virion interacts with specific receptors sites on hepatocytes leading to attachment and binding by means of the PreS1 domain, and subsequently penetrates the hepatocytes. It then UN coats, releasing partially double-stranded relax circular DNA into the cytoplasm. This is then transported into the nucleus and cellular enzymes synthesize DNA to complete the uncompleted strand converting it to covalent closed circular DNA (ccc DNA). The ccc DNA serves as the template to produce HBV messenger ribosomal nucleic acids (mRNAs) including a 3.5-kb RNA pre genome. (Beck and Nassal, 2007).

The pre genome and a viral polymerase protein (with HBV reverse transcriptase and RNase H activity) are encapsulated forming newly synthesized core particles. Using the RNA pre genome as template, the reverse transcriptase synthesize the negative strand within the capsule while the RNase removes the RNA pre genome template (Beck and Nassal, 2007). A complementary strand to the negative strand (positive DNA strand) is synthesized but the synthesis does not proceed to completion within the core, resulting in replicative intermediates consisting of full-length minus DNA strand and a positive DNA strand of variable-length about 20%–80% complete. Core particles (nucleocapsids) containing these DNA replicative intermediates with a relaxed circular DNA may bud from pre-Golgi membranes (acquiring HBsAg in the process), exiting the cell as a virion (Ganem and Alfred, 2004).

2.4 Pathogenesis of HBV infection

HBV after entering the body through a broken skin or mucous membrane, is transported to the liver which is the preferred site to cause infection. The Hepatic damage as a result of HBV infection is reported to be due to the response of the immune system of the body to the infection, as the virus itself is not directly cytopathic to infected liver cells (Xuanyong, 2011). There are two types of immune response which occur as a response to HBV infection. These are the innate and the adaptive immune response. The innate immune is an immediate and the first line response to infections. The innate immune response often results in the stimulated production of interferon alpha and beta by the infected cells. The interferon stimulates the expression of large quantities of interferon inducible genes (ISGs), which in turn initiate different kinds of intracellular antiviral pathways capable of limiting viral production and spread and ultimately minimizing pathogenic processes (Alexopoulou, *et al*., 2001).

Also, the hepatitis B virus can evade innate immune response shortly after infection of host cells by acting as a stealth virus as it spreads through the liver. It is able to do so by not stimulating any cellular gene expression including ISGs, The adaptive immune response is responsible for the elimination of infected viruses during HBV infection. It causes collateral damage to hepatocytes leading to hepatic injury and damage. The adaptive immune response is more pronounced in acute HBV infections and to a lesser extent in some chronic infections. (Wieland *et al*., 2004).

2.5 Clinical Presentation of HBV Infection

Primary hepatitis B viral infection is often accompanied with subclinical illness or with acute liver inflammation which may be mild or severe and may result in chronic infection (Robinson, 1995). About 90% of primary adult HBV infections result into acute hepatitis. The rest 5 - 10% of primary adult infections and more that 90% of new-born and infant HBV infections result into chronic hepatitis B, which may live with the patient for his/her entire life (Hollinger and Liang, 2001).

2.5.1Acute HBV infection

Acute hepatitis B virus infection is mostly characterized asymptomatic short mild illness which mostly resolves undetected (Jake, 2009). The author added that only about 1/3 of adults with acute hepatitis B show mild clinical signs and symptoms such as nausea and fatigue. After exposure to HBV, acute hepatitis usually manifest in two to three months. The amount of the virus one is exposed to. To some extent, determines the duration of the incubation time of acute

hepatitis also a short pre-jaundice symptom like body aches, fatigue, fever, nausea, anorexia etc. follow the incubation period. This period is accompanied with a striking rise in ALT levels and also high levels of HBV DNA and HBsAg. This pre-icteric period is followed by jaundice, after lasting for a few days to about one week. The jaundice period last for about one to two weeks. During this period the amount of the virus decline, and as the jaundice resolves HBsAg and viral DNA disappear from the serum, although symptoms may continue for about a week or month (Jake, 2009).

2.5.2Chronic Hepatitis B

Chronic hepatitis B has a dynamic and a variable clinical course. Early during infection, HBe Ag, HBsAg and HBV DNA are usually present in high titers, and there are mild to moderate elevations in serum aminotransferase levels. Nevertheless , the disease activity can resolve with time, either with persistence of high levels of HBe Ag and HBV DNA (the immune tolerance phase) or with loss of HBe Ag and fall of HBV DNA to low or undetectable levels (inactive carrier state‖). Other patients continue to have chronic hepatitis B, although some lose HBe Ag and develop anti-HBe (HBeAg-negative chronic hepatitis B. (Jake, 2009).

2.6 Diagnosis of Hepatitis B

The diagnosis of HBV infection and its associated disease is based on a combination of clinical, biochemical, histological, and serologic findings (Jake, 2009). The HBV infections especially chronic infections usually present as asymptomatic and diagnosis based on clinical signs and symptoms could be missed. Acute hepatitis B presents symptoms of fatigue, nausea, abdominal pain, darkening of urine, skin rashes, arthralgias etc. Later, it develops into jaundice just like the other viral hepatitis (WHO, 2002). Chronic hepatitis B patients do not show many of the symptoms of acute HBV infection including jaundice until liver damage is advanced, hence such patients can remain undiagnosed for a very long time (WHO, 2002).

Biochemical test can detect elevated liver enzymes level, although it not specific to Hepatitis B only. Test for liver enzymes aminotransferases that i.e. Aspartate Aminotransferase (AST) and Alanine aminotransferase (ALT) is sensitive and one of the widely used blood tests for evaluating patients with hepatitis B. These enzymes are usually contained within the hepatocytes and are spilled into the blood stream when the liver is injured or scarred raising the levels of the enzymes

in the blood indicating liver damage (Pan and Zhang, 2005). The authors' added that Patients with acute hepatitis B can present very high AST and ALT levels in serum which fall to normal levels in succeeding weeks or months as the patient clears the virus and seroconvert. Patients with chronic hepatitis B however typically have normal to mild elevation of AST and ALT levels which could last for years in the immune tolerant phase of chronic infection (Pan and Zhang, 2005).

Molecular technique is the most sensitive and specific test for detection of HBV genome, Also the Histological techniques helps in the examination of diseased specimen for the detection of antigens and components associated the hepatitis B virus (Hollinger and Liang, 2001). The serological detection of HBV markers has proven to be rapid and useful for large scale screening of HBV infection. Clinically useful serological markers used in the diagnosis of HBV infection include: hepatitis B surface antigen (HBsAg) and antibody to HBsAg (anti-HBs); antibody (anti-HBc IgM and anti-HBc IgG) to hepatitis B core antigen (HBcAg); and hepatitis B envelope antigen (HBeAg) and antibody to HBeAg (anti-HBe) (WHO, 2002). HBsAg can be detected in the serum from several weeks before onset of symptoms to months after onset. HBsAg is present in serum during acute infections and persists in chronic infections (Hollinger and Liang, 2001).

2.6.1 Hepatitis B Virus Serological Markers

2.6.1.1 The hepatitis B surface antigen (HBsAg):

This is the major component of the coat of the hepatitis B viral particle, coded for by the S gene weighing about 24 kilo Daltons and May or may not be glycosylated. The product of the S gene constitutes the major protein of HBsAg. Products of the PreS1 and PreS2 incorporated into the HBsAg are in small quantities in the shells of the non-infectious HBV particles (Mahoney, 1999). HBsAg can be detected in the serum from several weeks before onset of symptoms two months after onset. This marker can be detected early in the serum and it stays for a long time during the course of the HBV infection. (Hollinger and Liang, 2001). It has served as a useful diagnostic marker for rapid detection and screening on large scale for HBV infection.

2.6.1.2 The Hepatitis B core antigen:

This antigen is a component of the nucleocapsid, essential for the packaging of the virus and coded for by the C gene in the L- strand and weighing 18-19 KD. When transcribed, it usually moves into the endoplasmic reticulum where it gets cleaved. It is usually difficult to locate this antigen in serum by conventional methods but can be easily identified in liver biopsy of patients where it appears in the nuclei of infected hepatocytes (Eligouhari *et al*., 2008).

2.6.1.3 The Hepatitis B enveloped antigen:

This antigen is coded for by the C gene and translated from the same gene as HBcAg weighing 15.5 *k*D. This is a soluble antigen in the blood created by the pre-mature proteolytic cleavage of the core antigen. The presence of this antigen indicates that the virus is rapidly replicating in the patient and thus high numbers of virions are present in the blood; hence, the patient at this stage is highly infectious (Eligouhari *et. al.,* 2008).

2.6.1.4 Hepatitis B surface antibody:

This is a protecting and neutralizing antibody produced in response to infection with the HBsAg. The presence of this antibody against the common epitope ―a‖ of one HBsAg subtype confers immunity against re-infection or new infections with other subtypes (Zukerman, 2006). When this antibody develops after the HBsAg has been cleared from the serum at about six months after primary or acute infection, the antibodies persist for life (Mahoney, 1999).

2.6.1.5 Hepatitis B core antibody:

This antibody is produced by the body in response to infection with the Dane particle and can be detected just after the appearance of HBsAg. Detection of the hepatitis B core Immune globulin M (HBc IgM) is an indication of acute infection with Dane particle but the absence of anti-HBc-IgM and the presence of hepatitis B core Immune globulin G (HBc-IgG) together with HBsAg is an indication of chronic infection (Zukerman, 2006).

2.6.1.6 Hepatitis B enveloped antibody:

This marker develops after HBeAg a soluble protein in the core has been cleared from the blood of infected person. The presence of HBeAb in the blood of persons infected with HBV indicates that the person in less infective; thus, the number of virions in the blood are fewer (Zukerman, 2006).

2.7 Transmission of HBV Infection

Human is the only natural reservoir of human HBV (Robinson, 1995). There are four major contact modes of transmission of HBV includes: from mother to child (vertical/perinatal), non-sexual contact with an infected person (horizontal), including household transmission, sexual contact, and parenteral exposure to infected blood and other bodily fluids . HBV can survive outside the body for about seven days and hence can also be transmitted via contaminated inanimate objects like toothbrushes, baby bottles, toys, razors, eating utensils, hospital equipment and other objects, by contact with mucous membranes or open skin breaks. No evidence exists of an air-borne infection of HBV (Hollinger and Liang, 2001).

2.8 Treatment of Hepatitis B

Treatment for acute HBV infection is generally supportive. However, there is antiviral therapy for chronic hepatitis B but these antiviral treatment do not eradicate the HBV. The aim of treatment for chronic HBV infection is to reduce the risk of developing chronic liver disease by sustained suppression of HBV replication in the liver. Long-term treatment with antiviral drugs has been shown to be effective in reducing the risk of both disease progression and of developing hepatocellular carcinoma, by up to 50% (Lok and McMahon, 2007). The usual markers of successful therapy are the loss of HBeAg, seroconversion to anti-HBe antibodies, and reduction of circulating viral load. These are useful indicators, since patients with stable seroconversion to anti-HBe positive status typically have improved histologic findings in the liver, and this improvement tends to be maintained over the long term. Treatment regimens include administration of Interferon alpha which act as an immunomodulator, Lamivudine and Adefovir which are nucleotide analogues and directly block replication of the HBV genome (Ganem and Alfred, 2004).

2.9 IRON HOMEOSTASIS

Iron homeostasis requires coordination between tissues that export iron into plasma (duodenal mucosa, macrophages) tissues that utilize iron (mainly red blood cell precursors), and tissues that store iron (such as hepatocytes, pancreatic cells and cardiac cells). The iron storage protein, ferritin, reflects iron stores in normal conditions, but not in the case of inflammation or liver damage. The amount of iron in an average adult is 3–4 g. To support erythropoiesis and other metabolic processes about 25 mg iron/ day is needed. Aged erythrocytes stand for the predominant contribution, and only 1-2 mg of dietary iron is absorbed from enterocytes in normal conditions, equaling the amount of daily loss. Iron is distributed through blood plasma, where it is bound to the iron transport protein transferrin.

The body is dependent on regulation of the dietary uptake of iron, since losses are not modulated by iron excess or deficiency. The small peptide hepcidin is the master regulatory hormone of systemic iron metabolism. It is expressed in the liver and inhibits iron recycling from macrophages and enterocytes, by binding to and inducing the degradation of the cellular iron exporter ferroportin, thus lowering iron levels in serum. Consequently, deficiency of hepcidin will lead to iron overload. (Coimbra *et al*, 2013). Maintenance of iron metabolism within the physiological range is essential to human health. The liver synthesizes several proteins that are required for iron metabolism in addition to iron storage. Chronic liver injury and histological alteration impact iron metabolism and altered iron metabolism may aggravate liver injury. For instance, both experimental and clinical studies have suggested that sustained iron retention in hepatocytes aggravates liver injury and is associated with higher risks of developing fibrosis, cirrhosis, and HCC in CHB patients (Jaeschke et *al*, 2002, Mao *et al*, 2015).

2.10 IRON TOXICITY

In switching between its ferric (FeIII) and ferrous (FeII) form, iron has the ability to easily donate and accept electrons. This makes iron essential for various processes, most importantly those of oxygen transport. On the other hand iron can also be harmful. To prevent its harmful effects, iron is bound to transferrin in the circulation and stored by ferritin. In normal conditions there are hardly any notable levels of free or labile iron. In the case of iron overload disorders, free iron catalyzes the production of highly toxic hydroxyl radicals. Antioxidant defense mechanisms counterbalance

this process, but as iron overload increases, they become insufficient.10-12 in hemochromatosis massive iron overload may cause cell death and the initiation of fibrogenesis. If the excess iron is not removed there can be a progress to cirrhosis (Deugnier *et al*, 1992).

2.11 Iron metabolism and HBV infection:

Viral infections that disrupt liver function can be accompanied by changes in iron homeostasis, and iron loading of this organ can exacerbate chronic viral disease. Recent studies have revealed that the liver plays an important role in iron homeostasis by secreting a peptide hormone named hepcidin. This hormone synthesis, which occurs predominantly in the liver hepatocytes, is integral to maintaining iron homeostasis in the body. Hepcidin binds to the cellular iron export channel ferroportin to cause ferroportin internalization and degradation thereby de-creasing iron efflux from enterocytes and macro-phages into plasma. Hepcidin expression is up regulated by excess iron or inflammation, whereas increased erythropoiesis and reduced iron stores all down regulate hepcidin expression (Wang et al 2013).

Chronic viral hepatitis are often associated with iron overload, which is also a major risk factor for HCC development. This may be related to the ability of iron to generate oxidative stress, leading to tissue damage and chronic inflammation in the liver (Wang et al 2016).cellular operations, including DNA synthesis and the generation of ATP, require iron. Viruses attack cells in order to replicate, and efficient replication needs an iron-replete host. Some viruses selectively infect iron-acquiring cells by binding to transferrin receptor 1 during cell entry. Other viruses alter the expression of proteins involved in iron homeostasis, such as HFE and hepcidin (Drakesmith et al 2008).

Patients with iron overload (genetic or secondary forms of hemochromatosis) are at increased risk for the development of severe infections by such organisms.Conversely, patients with infections or other inflammatory conditions show a decrease in serum iron concentration (hypoferremia of infection), largely due to the effects of interleu-kin-1, an important mediator of the inflammatory response. The hypoferremia caused by interleukin-1 (and perhaps other factors) is believed to be a host defense mechanism to help limit infection. The iron-binding proteins, lactoferrin and transferrin, by virtue of binding iron with great avidity, also play important roles in resisting infections. They diminish the availability of iron to pathogenic organisms. Lactoferrinis found in

colostrum and mucosal secretions, where it plays a bacteriostatic role, and in specific granules of polymorphonuclear leukocytes, where it is thought to aid in phagocytosis and killing of invading microorganisms. Exocytosis of lactoferrin from neutrophils during the inflammatory response may further stimulate antimicrobial actions of monocytes and macrophages. Transferrin, the major iron-binding protein of the blood, is normally only about one-third saturated with iron, and thus limits iron availability to invading microorganisms (Mocchegiani, et al 2012).

2.12 Previous study

Mao et al (2015) found when Comparing no cirrhotic patients and healthy controls, the serum transferrin of cirrhotic patients was lower and the serum iron and ferritin values were higher (P<0.001, all). In cirrhotic patients, the serum iron and ferritin levels correlated positively with serum alanine transaminase levels and the transferrin levels were inversely related to both end-stage liver disease scores and iron levels (all P<0.01).Serum iron markers tended to be aberrant in chronic HBV-infected patients with cirrhosis. The liver injury associated with HBV infection, but not chronic HBV infection directly, is likely the main cause for iron metabolism disorder.

Sebastiani et al (2012) conduct study to investigate hepatic iron deposits and serum iron indices in 205 consecutive patients with hepatitis B and compensated liver disease. Mean age of the patients was 42.4 ± 12.4 years and 72.5% were males. Coinfection with hepatitis delta virus (HDV) was present in 8.8%. At least one of the serum iron indices was elevated in 41.5% of cases. Hepatic iron deposits were detected in 35.1% of patients, most of them being minimal (grade I) (59.7%) or mild (grade II) (27.8%). Variables significantly associated with hepatic iron deposits were male gender (P = 0.001), serum ferritin (P = 0.008), γGT (P = 0.05) and alkaline phosphatase (P = 0.05) levels In conclusion, in well□compensated chronic hepatitis B infection, hepatic iron deposits and elevation of serum iron indices are common, especially in male gender and in patients coinfected with HDV. As HBV/HDV liver disease is generally more rapidly progressive than that caused by HBV monoinfection, we speculate that iron overload may be one of the factors contributing to the severity of liver disease.

Wang et al (2013) found that Serum IL-6, ferritin, and hepcidin levels were significantly higher in patients with hepatitis B and in HCC patients than in controls *($P<0.05$)*, and strong positive correlations were found between hepcidin and ferritin, AST, ALT, GGT, ALP, TBIL, IBIL, and

AFU, as well as between log [hepcidin] and log [HBV], respectively. There were no significant differences in hematological parameters, including WBC, Hb, and platelets among hepatitis B patients, nor was a correlation found between hepcidin and any hematological parameters. Our results indicate that hepcidin expression is regulated by iron and inflammatory factors in hepatitis B infection patients, and that the virus load can affect hepcidin production.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1Study design:

This is case - control study conducted to investigate Iron parameters in different HBV viral load Sudanese patients.

3.2 Study area:

This study was conducted at EL-Rayadi Medical Laboratory Khartoum –Sudan during the period from April to June, 2019.

3.3 Study population and sample size:

One hundred and twenty subjects were included in this study sixty patients with HBV infection as case group sixty subjects as healthy control group. Case group were included in this study according to formula

$$n = \frac{N}{1+ (N) (e)^{2}}$$

N = total population of HBV Patients (200) in AL-Rayadi Medical Laboratory

E = error estimation (this study uses 0.05)

n = 60

3.4 Sampling technique:

Participants were selected through simple random sampling technique that technique give equal chance to all patients with HBV attending EL-Rayadi Medical Laboratory during study period to be included in this study.

3.5 Study variables:

3.5.1 Dependent variables:

Serum iron, TIBC, transferrin and ferritin.

3.5.2 Independent variables:

Viral load activity and ALT.

3.6 Inclusion criteria:

Patients who diagnosed with HBV.

3.7 Exclusion criteria:

Patients who not suffering from any health status that affect iron metabolism.

3.8 Laboratory analysis:

Blood samples were collected from all patients and controls, Serum iron, Transferrin, total iron binding capacity and ALT were measured with an automatic clinical chemistry analyzer (DIRUI CST 240) (Appendix C).

-iron principle: Transferrin-bound ferric ions in the sample are released by guanidinium and reduced to ferrous by means of ascorbic acid. Ferrous ions react with ferrozine forming a colored complex that can be measured by spectrophotometry. (Appendix E)

- Transferrin in the sample precipitates in the presence of anti-human transferrin antibodies. The light scattering of the antigen-antibody complexes is proportional to the transferrin concentration and can be measured by turbidimetry. (Appendix D)

-TIBC: Excess of Fe^{3+} is added to the sample to saturate serum transferrin. Uncomplexed Fe^{3+} is precipitated with magnesium hydroxide carbonate and the iron bonded to protein in the supernatant is then spectrophotometrically measured. (Appendix G)

Ferritin was measured by using immunoassay system (TOSOH AIA1800). (Appendix F)

HBV DNA in serum results was collected from record data using real time PCR. (Appendix A)

The case group was classified according to the reference ranges very low: less than 10.000 IU/ml, low: 10,000-100.000 IU/ml and high: 1000000-10000000 IU/ml

3.9 Statistical analysis:

Mean and standard deviation, frequencies and percentage for the data analysis of the background of the data.

The collected data was computerized into Excel format for easy data retrieving through the statistical package for social science (SPSS. version 23). The data was already clean and checked through SPSS which was used for descriptive statistics and statistical procedures. independent samples T tests were used on numerical data to look at comparing mean of iron profile between

case and control and one way ANOVA were used to compare mean of iron profile between different viral load activity in case group also person correlation to correlate between ALT and viral load level. Decision rule was set based on a p-value set at 0.05 with 95% confidence interval and whenever the p-value was less than 0.05 the null hypothesis was rejected and the alternative hypothesis was considered. The data presented as mean ±SD.

3.10 Ethical consideration

The objective of the study was explained to all individual participants in this study and verbal informed consent was obtained from all participants, Participant has right to withdraw at any time without any deprivation, Participant has right to no harm (privacy and confidentiality), Participant has right to benefits from the research knowledge and skills. And Study data/information was used for the research purposes only. The privacy issues was intentionally considered.

CHAPTER IV

RESULTS

Chapter IV

Results

4.1 Demographical data

This is case control study was designed to determine the levels of iron profile in hepatitis B virus patients.120 subjects with mean age (47±11) years were included in this study, 60 patient's diagnosed with hepatitis B virus infection and 60 as healthy control subjects were included in the study. The results are expressed as mean and standard deviation (SD) for the continuous variables of normally distributed data.

Hepatitis B virus infected patient were classified according to level viral overload activity results to very low, low and high. About 53.4% of study participant were female which among of them 45% in case group and 61.6 in control group, also about 46.6 of study participant were males which among of them 55% in case group and 38.3 in control group

Table 4.1: Demographic data of the patients and controls

	Case groups according to viral load			**Control group**
	very low (n=17)	low (n=25)	high (n=18)	
Age (years)	45±7	49±11	45±9	49±13
Gender (F/M)	7\10	13\12	7\11	37\23
Duration (years)	1±0.5	2±0.5	5±1.5	-

F\M; female male ratio

4.2 Serum Iron profile among case and control

Serum iron (SI) and TIBC levels were significantly lower in case group patients when compared to control group (p. value 0.000).Ferritin level were significant higher in case group when compared with control group (p. value 0.000). And no significant differences in serum transferrin level**.**

Table 4.2: mean of iron profile among case and control

	Case (mean± SD)	Control (mean ±SD)	P value
Serum iron (µg/dl)	86.3±36	129 ±49	0.00
TIBC (µg/dl**)**	212.6±56	374±46	0.00
Transferrin (mg/dl)	174.9 ±59	177.1±53	0.145
Ferritin (µg/ml)	241.6±161	86.6±13	0.00

4.3 Serum iron parameters between different viral load activity level

Iron parameters were compared between case groups (classified according to level of viral load) found that serum iron and TIBC decreased in the patient with high viral load p. value (0.000) when comparing with low and very low that mean this parameters decreased associated with increase viral activity while the ferritin increased in patient with high viral load when comparing with low and very low p. value (0.000) which reflect liver damage severity. Transferrin insignificantly decreased p. value (0.191) in very low when comparing with other levels**. Table 3**

Table 4.3: means of iron profile according to viral load activity

	Very low	Low	High	P value
Serum iron (µg/dl)	98±23	87 ±43	82±26	0.000
TIBC (µg/dl**)**	223±65	205±45	193±35	0.000
Transferrin (mg/dl)	173 ±45	177 ±61	177±74	0.191
Ferritin (µg/ml)	161±61	198±54	212±54	0.000

4.4 Correlation between viral load level and ALT enzyme

ALT which reflect the liver damage were significantly had negative correlation with viral load level with (p. value 0.000)

Table 4.4 Correlation between viral load level and ALT enzyme activity

	R	**P .value**
viral load level	-.515	0.000

*r= correlation confession

CHAPTER V

DISCUSSION, CONCLUSIONS AND RECOMMINDATIONS

CHAPTER V

DISCUSSION, CONCLUSIONS AND RECOMMINDATIONS

5.1 Discussion:

In this thesis, study was conducted to characterize and evaluate the iron profile in HBV infected patients in Khartoum state and found that Serum iron (SI) and TIBC levels were significantly lower in study group when compared to control group, this suggestion supported with study done by Gao *et al* (2018) Serum iron, total iron binding capacity, significantly lower in patients with cirrhosis and hepatocellular carcinoma, whereas the hepcidin level was higher than that in chronic hepatitis B patients ,Naciye et al (2011) disagreed with this finding and found that Serum iron (SI), TIBC, ferritin, and TS levels were statistically not different in controls and chronic hepatitis cases.

Ferritin increased in patients with hepatitis B virus when comparing with control group, This suggestion supported with study done by Wang et al (2013) found that Serum ferritin, and hepcidin levels were significantly higher in patients with hepatitis B and in HCC patients than in controls *($P<0.05$)*. Indicated that Hepcidin expression was regulated by iron and inflammatory factors in HBV infected patients, and that the virus accumulation in infected hepatocytes can affect Hepcidin production. Also Gao et al (2018) found that The serum Ferritin level was significantly higher in hepatitis B-related liver diseases reflecting the increased release of Ferritin from hepatocytes that had increased iron deposition and were destroyed as a result of HBV replication and/or iron retention-related injury This suggestion was supported by our finding that the serum Ferritin level was higher in HBV patients because a more prominent liver injury, as indicated by ALT level.

The serum transferrin level shows no significant difference in study group when comparing with control group, Gao et al (2018) was disagreed with this finding, and they found that serum transferrin levels were significantly lower in patients with cirrhosis and hepatocellular carcinoma. According to reference range of viral load this study found that, serum iron and TIBC decreased in the patient with high viral load when comparing with low and very low that mean this parameters

decreased associated with increase viral activity, while the ferritin increased in patient with high viral load when comparing with low and very low which reflect liver damage severity which detected by ALT level. Transferrin insignificantly decreased in very low when comparing with other levels**.** Wang et al (2016) Found that Correlation analysis indicated that serum hepcidin was negatively correlated with HBV-DNA load, as we know the Hepatocytes produce and secret Hepcidin, an acute phase reactant protein that may negatively regulate the endogenous iron level and reduce the release of iron from cells by interacting with the cellular iron exporter ferroportin that leads to subsequent internalization and degradation that explore the reduce in serum iron and TIBC Ganz, (2015).

ALT which reflect the liver damage were significantly had negative correlation with viral load level.

5.2 Conclusions:

According to the finding this study conclude that, Iron metabolism disorders can occur in patients with HBV infection, Serum iron and TIBC levels were significantly lower in case group patients when compared to control group, Ferritin level was significant higher in case group when compared with control group. The serum markers of iron metabolism disorders vary in different level of HBV-activity levels, found that serum iron and TIBC decreased in the patient with high viral load when comparing with low and very low that mean this parameters decreased associated with increase viral activity while the ferritin increased in patient with high viral load when comparing with low and very low which reflect liver damage severity.

5.3 Recommendations:

Measurement the iron parameters regularly in the patient with hepatitis B to avoid iron metabolism disorder.

Liver biopsy should be performed in patients to hepatitis B that help in diagnosis of hereditary hemochromatosis.

Make this research with larger sample size and included all hepatitis virus type infection and all causes of liver disease.

Measurement of hepcidin level because it is the major regulatory iron protein.

CHAPTER VI

REFRENCES

CHAPTER VI

REFRENCES

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Appendices

Appendix A

Real time PCR

Appendix B

Tosoh AIA 1800

Appendix C

DIRUI CS-T248