بسم الله الرحمن الرحيم

Sudan University of Science and Technology
College of Graduate Studies

Sero-diagnosis of Hepatitis B Virus Surface Antigen amongPregnant Women in Khartoum State

التشخيص المصلي للمستضد السطحي لفيروس إلتهاب الكبد الوبائي لدى النساء الحوامل في ولاية الخرطوم

A dissertation submitted for partial fulfillment of the requirements of M. Sc. degree in Medical Laboratory Science (Microbiology)

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2016

الآية

بسم الله الرحمن الرحيم

قال تعالى:

وَمِنَ النَّاسِ وَالدَّوَابِّ وَالْأَنْعَامِ مُخْتَلِفٌ أَلْوَانُهُ كَذَٰلِكَ ۗ إِنَّمَا يَخْشَى اللَّهَ مِنْ عِبَادِهِ الْعُلَمَاءُ ۗ إِنَّ اللَّهَ عَزِيزٌ غَفُورٌ

صدق الله العظيم

سورة فاطر: الآية (28)

DEDICATION

To my mother, father and brothers

ACKNOWLEDGMENT

All praise is due to **ALMIGHTY ALLAH**.

I have insufficient words to express my gratitude to my supervisor **Prof. Humodi Ahmed Saeed** for his priceless inspiration and encouragement from the very beginning has been wonderful experiences. His comments and suggestions encourage me to think critically.

I am grateful to my brother Ammar for his assistance, my teacher and colleagues in the Department of Microbiology, Sudan University of Science and Technology for help and fruitful discussion.

Finally, I would like to thanks my family for their warmth love and confidence.

ABSTRACT

Hepatitis B virus (HBV) is a leading cause of liver disease worldwide. There is much data exist concerning HBV during pregnancy in many African countries, but nopublished data are available in Sudan. The objectives of this study were to investigateHBVamong as well as possible risk factors among pregnant women.

Blood specimens were collected from pregnant women attended Omdurman Maternity Hospital in Khartoum State. Sera were separated by centrifugation.Participated women were interviewed by pre-structured questionnaire. Enzyme Linked Immunosorbent Assay (ELISA) technique was use todetect of Hepatitis B Virus surface antigen (HBVsAg).

Ninety (n=90) pregnant women were participated in this study. The age of the participants ranged from 15 to 45 years old. The mean age was 24 years. Most 56(62.2%) the participants fall in age ranged from 26 to 35 (%). The majority 53 (58.9 %)of the participantswere from Khartoum City, the rest 37 (30.1%) were distributed as follows: Khartoum North 20 (21.8 %), Omdurman 9 (9.8%) and out of Khartoum State 8 (9.5%).Of the investigated women, 59(65.6%) of the werenot vaccinated while 31(34.4%) vaccinated. 77 (85.6%) were house wife and only 12 (14.4%) employees. Study on detection of HBVsAg revealed that 5(5.6%) were positive, and the rest 85(94.4%) were negative.

It is concluded that high percentage of pregnant women were infected with HBV.

Significant ($P< 0.05$) relation was noticed between HBV infected and dental manipulation

as well as blood transfusion. The study indicated insignificant ($p<0.05$) association betweenHBV infection and risk factors, including residence, age occupation and gestational age. Further studies with large number of participants are required to validate the result of this study.

الخلاصة

فيروس التهاب الكبد الوبائي ب من مسببات أمراض الكبد ويعتبر من أهم المشاكل الصحية في كل من الدول المتقدمة والنامية. تتوفر بيانات كثيرة بخصوص الالتهاب الفيروسي أثناء فترة الحمل في معظم الدول الأفريقية ولكن البيانات المتوفرة في السودان قليلة بخصوص البحث لدراسة التهاب الكبد الوبائي ب بين النساء الحوامل بالإضافة للعوامل.

تم جمع عينات الدم من النساء الحوامل من مستشفى الولادة بأمدرمان ولاية الخرطوم. تم إجراء استبيان للنساء المبحوثات. تم جمع عينات الدم لفصل الأمصال بواسطة جهاز الطرد المركزي(ELISA). تم ضبط(ELISA) لدراسة المستضد السطحي لالتهاب الكبد الوبائي ب.

تراوح أعمار المبحوثات ما بين 10-45 عام المتوسط 24 عام. معظم المبحوثات 56 (62.2%) في عمر يتراوح ما بين 26-35 سنة، الأغلبية 53 (58.9) من ولاية الخرطوم والباقين من الخرطوم بحري 20 (21.8%)، 9 (9.8%)، 8 (9.5%) على التوالي.

59 (65.6%) من المبحوثات لم يأخذن الفاكسينات و 31 (34.4%) منهن أخذن الفاكسينات، 77 (85.6%) من المبحوثات ربات بيوت وفقط 12 (14.4%) عاملات.

أظهرت الدراسة أن ما بين (90) من الحوامل فقط 5 (5.6%) أظهرن نتيجة موجبة HBVsAg. الباقي 85 (94.4%) أظهرن نتيجة سالبة.

وجدت علاقة معنوية ($P<0.05$) ما بين الإصابة وعلاجات الأسنان ونقل الدم. وهنالك ارتباط غير معنوي ما بين التحصين، السكن، العمر، والعمر الحملي.

خلصت الدراسة أن نسبة عالية من التهاب الكبد الوبائي وجدت لذلك لا بد من وجود برنامج تعليمي صحي للنساء الحوامل. وأوصى بإجراء مزيد من الدراسات مع عدد كبيرمن العينات للتحقق من صحة نتائج هذه الدراسة.

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CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1 Introduction

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

Hepatitis B virus (HBV) is a leading to cause of liver disease.Approximately one-third of living persons have been infected with HBV at one point in their lives, and 350 million of them have become chronic HBV patients or carriers. More than 1 million people die each year due to HBV-related diseases, such as chronic active hepatitis, cirrhosis, and hepatocellular carcinoma(Ozer*et al.*, 2011).

Transmission of the HBV can occur through of several ways, by the intravenous rote e.g. by transfusion of infected blood or blood product, or by contaminated needles used by drug user (around 30%), tattooists or acupuncturists. By sharing objects that may have a very tiny amount of blood on them, such as a tooth brush, razor, or tools used for manicures. By close personal contact, such as during sexual intercourse, particularly in men having sex with men (homosexual), also heterosexual exposure and at the same time bisexuality (Kumar*and Clark.*, 2009).

Vertical transmission from mother to child in uterus (intrauterine), during parturition or soon after birth (prenatal) which constitute the major one in thiscategory, and from breast

feeding(Kumar*and Clark.*,2009). Horizontal transmissions occur particularly in children through minor abrasion or close contact with other children, and HBV can survive on household articles, e.g. toys, toothbrush, for prolonged period so transmission may be possible. HBV is present in high titer in blood and exudates (for example from skin lesions) of acutely and chronically infected persons (Kumar andClark., 2009).

Moderate viral titer are found in semen, vaginal, secretions and saliva, other body fluids that don't contain blood or serous fluids, such as feces or urine cannot as sources of HBV(Lung*et al.*,2008). It cannot be spread through sneezing, coughing, hugging or same risk for a hepatitis B infection but some groups are at high risk because their an occupation or life choices (Jawetez*et al,*2007).

Sudan is classified as endemic area of hepatitis B surface antigen (HBs Ag), seroprevalence ranging from 6.8% in central Sudan to high 26%4 in southern Sudan(Mudawi*et al.*, 2008).

1.2 Rationale

Hepatitis B virus is a major cause of liver disease worldwide and a potential cause of substantial morbidity and mortality in the future. The complexity and uncertainty related to the geographical distribution of HBV infection and chronic hepatitis B virus, determination of it associated with risk factors and evaluation of cofactors that accelerate its progression, under-score the difficulties in global prevention and control of HBV.

Study of the hepatitis among pregnant women group is very important to protect transmission of disease to children. Therefore, this study was aimed to study the hepatitis B surface antigen among pregnant women in Khartoum State and to identify possible risk factors associated with hepatitis B infection in them.

1.3Objectives

1.3.1 General objective

To study the Hepatitis B virus among pregnant women attending OmdurmanMaternityHospital in Khartoum State.

1.3.2 Specific objectives

a. To detect the Hepatitis B surface antigen (HBs Ag) in pregnant women.
b. To compare between HBs Ag and risk factor associated with HBV infection.

CHAPTER TWO

LITERATURE REVIEW

2.1 Hepatitis B virus (HBV)

2.1.1Morphology and structure

Hepatitis B virus is a member of the hepadnavirus family (Levinson., 2006). The virus particles, virions consist of an outer lipid envelope and icosahedral nucleocapsid core composed of protein. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity. The outer envelope contains embedded proteins which are involved in viral binding of, and entry into, susceptible cells (Sonnabend*et al.*, 2010). The virus is one of the smallest enveloped animal viruses with a virion diameter of 42nm and pleomorphic form exist including filamentous and spherical bodies lacking a core, these particles are not infectious and are composed of the lipid and proteins that form a part of the surface of the virion, which is called the surface antigen (HBs Ag) (Mandel*et al.*,2005).

2.1.2 Genome

Hepatitis B virus is a small DNA virus and belong to a group of hepatotropic DNA viruses (hepadnaviruses).The virus consists of nucleocapsid and an outer envelope composed mainly of three antigens (HBs Agthat play a central role in the diagnosis of HBV infection). The nucleocapsid contains HBc Ag, a DNA polymerase reverse transcriptase, the viral genome as well as cellular proteins (Seto*et al.*,2011).

The genome consist of partially double stranded DNA molecules of about 3200 base pairs in length with known sequence as well as genetic organization, the presurface1(pre-s1),pre-surface2(pre-s2) and the surface genes (S) code for the various HBs Ag. The protein encoded by pre core (pre-c), code gene (c) undergoes post transitional modification to yield hepatitis B envelope antigen (HBe Ag), which is a sero-marker for high viral replications the core gene codes for HBc Ag, the major structural protein nucleocapsid. Finally the X gene code the hepatitis B xAg. HBx Ag has been shown to be a potent trans activator for cellular and viral gene (Baumert*et al.*, 2007).

The viral DNA polymerase transcriptase is encoded by the polymerase gene(p) and is a central important for the viral replication. Different from all of mammalian DNA viruses, hepadnaviruses replicate via reverse transcription of a RNA intermediate, the pregenomic RNA, which is strategy central to the RNA retroviruses (Baumert*et al.*, 2007).

2.2Replication

The life cycle of the HBV is complex. Hepatitis B is one of the few known nonretroviralviruses which used reverse transcription as a part of its replication process. The virus gain entry in to the cell by binding to an unknown receptor on the surface of the hepatocytes and enter it by endocytosis. Because virus multiplies via RNA made by a host enzyme, the viral genomic DNA has to be transformed to the cell nucleus by host protein called chaperones. The partially double stranded viral DNA is then made fully double stranded and transform in covalently closed circular DNA (cccDNA) that serves as template, for transcription of four viral mRNAs. The largest mRNA, (which is larger

than the viral genome), is used to make the new copies of the genome the capsid core protein and the viral DNA polymerase. These four viral transcripts undergo additional processing and go on to form progeny virions which are released from the cell or returned to the nucleus and recycled to produce even more copies. The long mRNA is then transported back to the cytoplasm where the virion p protein synthesized DNA via its reverse transcriptase activity(Levinson., 2006).

2.3Serotypes and genotypes

For vaccine purposes HBV has one serotype based on HBs Ag, however, for epidemiological purpose there are four subtype of HBs Ag based on a group specific Ag (a) was marked and to sets of mutually exclusive based on these antigenic epitopes which are presented on its envelope proteins, and in to 8 genotype (A-H) according to overall nucleotide sequence variation of the genome.

The genotype has a distinct geographical distribution and is used in tracing the evaluation and transmission of the virus. Differences between genotype affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination. Genotypes differ by at least 8% of their sequences and were first reported in1988 when six were initially described (A-F). Tow further types have been described (G&H)(Baumert*et al.*, 2007).

2.4 Occult hepatitis B

Occult hepatitis B is defined by the presence of hepatitis B virus DNA in serum or liver in the absence of HBs Ag. Serum HBV level is usually less than 104 copies/ml. Although occult HBV infection has been identified in patient with chronic liver disease two decades ago. Occult HBV infection has been found in patient with hepatocellular carcinoma (HCC), past HBV infection, or chronic hepatitis C virus, and individual without hepatitis B serological markers, the frequency of the diagnosis depends on the relative sensitivity of HBV DNA assay and the prevalence of HBV infection in the population. Collectively around 30% to 35% of HBs Ag negative. Subject with chronic hepatitis with or without HCC have positive serum HBV DNA (range from 5% to 55%) (Mumtaz*et al,*2011).

The clinical significance of occult HBV infection remains unclear. Occult infection represents a potential transmission source of HBV via blood transfusion or organ transplantation. Furthermore some studies suggested that occult might affect responsiveness of chronic hepatitis C to interferon therapy and disease progress (Jinlin*et al.,* 2005).

2.5Mutants

Mutation occurs in the various reading frame of the HBV genome. These mutants can emerge in patient with chronic HBV infection (escape mutant) or can be acquired by infection. HBs Ag mutant are produced by alterations in the 'a' determinants of the HBs Ag proteins with usually a substitution of glycine for arginine at position 145. This result

in changes in the antibody binding domain andthe usual tests or HBs Ag may be affected. A mutation in the pre core region when a guanosine (G) to adenosine (A) change creates a stop codon (TAG), prevent the production of HBe Ag, but the synthesis of HBc Ag is unaffected. To detect infectivity, HBV-DNA must always be measured as no HBe Ag will be present. DNA polymerase mutant occur, particularly after lamivudine therapy (Kumar*et al.*,2009).

2.6 Physical properties

Unlike enveloped viruses, which are usually unstable and weak in horrible condition, HBV is a very strong virus tolerating extreme condition. The stability of HBs Ag does not always coincide that of the infectious agent. However, both are stable at -20^{o}C for over 20years and stable to repeated freezing and thawing. The virus also is stable at 37^{o}C for 60 minutes and remains viable after been dried and stored at 25^{o}C for at least one weak. HBV (but not HBs Ag) is sensitive to higher temperature 100^{o}C for 1 min, or to longer incubation period (60 or 10 hours) depending on amount of virus present in the sample. HBs Ag is stable at ph 2.4 for 8 up to 6 hrs, but HBV infectivity is lost, sodium hypochlorite 0.5% e.g. (1: 10 chloride bleach) destroys antigenicity within 3 min at lost protein concentration, but undiluted serum specimen require higher core (5%). HBs Ag is not destroyed by ultraviolet irradiation of plasma or other blood products and viral infectivity may also resist such treatment (Ozer*et al.*,2011).

2.7High risk groups

The health care workers (HCWs) and medical students are at risk of infection with HBV through occupational exposure to blood and infection body fluids and HBV is also the most easily transmitted blood borne pathogens (Amini*et al.*,2005).

The following groups represent the high risk group(Amini*et al.*, 2005):

a. Infants from mother who are infected at the time of delivery.
b. Partners or individuals living in close house hold contact with an infected person.
c. Individual with multiple sex partner (past or present).
d. Individual who has been diagnosed with a sexually transmitted disease.
e. Illicit drug user (injection, inhaling, snorting, popping pills).
f. Men who have sex with men.
g. Individual who receive a blood transfusion.
h. Individual who get tattoos or body piercing.
i. Individual who travel to countries where HBV is common.
j. Individual with early kidney disease or undergoing kidney dialysis.
k. Individual who use blood products for medical conditions (i.e.hemophilia).

2.8 Epidemiology

HBV is worldwide health care problem, approximately 350 million people are lifelong carrier, and most of them are in Asia, in USA, an estimated 200.000 new cases occur

annually. It has an effective vaccine which reduces the incidence rate in same area like Taiwan, future problem with HBV is merging mutant strain which may abort the eradication of the virus (Mandel*et al.,* 2005).

The USA have a lower carrier rate (0.5-2%), but it rises to 10-20% in some parts ofAfrica, the middle, and the east (Kumar*and Clark.,* 2004). The prevalence of HBV infection varies markedly throughout regions of the world.

Hepatitis B is highly endemic in developing regions with large population such as south East Asia, China, Sub-Saharan Africa and the Amazon Basin, where at least8% of the population HBV was chronic carrier. In these areas, 70-95% of thepopulation shows post or present serological evidence of HBV infection. Mostinfections occur during infancy or childhood. Since most infections in children areasymptomatic, there is little evidence of acute disease related to HBV, but the ratesof chronic liver disease and liver cancer in adult ever high (Jinlin*et al.*,2005).

The endemicity of HBV is low in most developed area, such as NorthAmerica, Northern and Western Europe and Australia(Jinlin*et al.,* 2005).In these regions, HBV infects 5-70% of the population, and only 0.5-2% ofthe population are chronic carriers. In these areas, most HBV infections occur in adolescents and young adults(Jinlin*et al.,* 2005).

2.9 Pathogenesis

Among all viral hepatitis, the immune-pathogenesis of hepatitis B has beenstudied most extensively. The existence of inactive hepatitis B carriers with normal10liver histology

and function suggest that the virus is not directly cytopathic. Thefact that patients with defects in cellular immune competence are more likely to remain chronically infected rather than to clear the virus is cited to support the role of cellular immune responses in the pathogenesis of hepatitis B related liver injury.

The model that has the most experimental support involves cytolytic T cells sensitized specifically to recognize host and hepatitis B viral antigens on the liver cell surface (Ganem*et al.,* 2004).

Laboratory observations suggest that nucleocapsid proteins (HBc Ag andpossiblyHBe Ag), present on the cell membrane minute quantities are the viral target antigens that invite cytolytic T cells to destroy HBV(Ganem*and Alfred.*, 2004).

Although the precise mechanism of liver injury in HBV infection remain elusive, studies of nucleocapsid proteins have shed light on the profound immunologic tolerance to HBV of babies born to mothers with highly replicative(HBe Ag positive), chronic HBV infection. In HBe Ag, expressing transgenic mice, In utero exposure to HBs Ag, which is sufficiently small tolerance to both nucleocapsid proteins(Ganem*and Alfred.*,2004).

Four different stages have been identified in the viral life cycle of hepatitis B: The first stage is immune tolerance. The duration of this stage for healthyadults is approximately 2-4 weeks and represent the incubation period. For newborn, the duration of this period is often decades. Acute viral replication is knowntocontinue despite little or no elevation in the amino transfer's levels and nosymptoms of illness (Ganji*et al.*,2011).

In the second stage, an inflammatory reaction with a cytopathic effectoccurs. HBe Ag can be identified in the sera, and a decline of the level hepatitis Bvirus (HBV) DNA is seen. The duration of this stage for patient with acuteinfection is approximately 3-4 weeks (symptomatic period). For patient withchronic infection, 10 years or more may elapse before cirrhosis develops (Ganji*et al.*,2011).In third stage, the host can target the infected hepatocytes and the HBVviralreplication no longer occurs, and HBeAb can be detected (Ganji*et al.*,2011).

The HBV DNA levels are lower or undetectable, and amino transferase levels arewithin the references range- in this stage, an integration of the viral genome intothe host's hepatocytes genome takes place. HBs Ag still present (Ganji*et al.*,2011).

In the fourth stage, the virus cannot be defected and antibodies to viralantigens have been produced. Different factors have been postulated to influencethe evolution of these stages, including age, sex, immunosuppressant, and co-infectionwith other viruses (Ganji*et al.*, 2011).

Recent experiments suggest that some of the inflammatory by product, notably interferon g (INF g) and TNFα can have antiviral effects that do notinvolve killing of the target cells. When cytotoxic T lymphocytes are transferred tomice that bear replicating HBV, viral DNA and RNA throughout the liver rapidly disappear, even from viable, uninjured hepatocytes, an effect that can be blocked by the administration of Abs to TNFα and IFNg. Such non cytocidal antiviral effects may be important for viral clearance in natural infection. In fact, cytokine release triggered by unrelated hepatic infection in HBV.

Transgenic mice can have the same effect(Ganji*et al.*,2011).This phenomenon may explain the suppression and occasional clearance of chronicHBV infection in patient with superimposed acute hepatitis caused by unrelatedviruses(Ganji*et al*,2011).

2.10 Clinical features

The prodromal symptoms of viral hepatitis are systemic and quite variable.Constitutional symptoms of anorexia, nausea and vomiting, fatigue, malaise,arthralgias, headache, photophobia, pharyngitis, cough, and coryza may precedethe onset of jaundice by 1to 2 weeks (Kumar*and Clark.*, 2009).

Symptoms resulting from acute hepatitis B infection among adult are common,with jaundice occurring approximately 12 weeks after initial infection (Kumar and Clark., 2009).Chronic active hepatitis follows hepatitis B infection in 3% of cases.Many people with a chronic hepatitis B remain symptom free for up to 30 years,but others experience ongoing symptoms similar to those of acute hepatitis B.chronic hepatitis B is a serious disease that can result in long –term healthproblems (Kumar*and Clark.*,2009).Rarely, a few spider angiomas appear during the icteric phase and disappearduring convalescence (Kumar*and Clark.*, 2009).

During the recovery phase, constitutional symptoms disappear, but usuallysome liver enlargement and abnormalities in liver biochemical test are still evident(Ganem*and Alferd.*, 2004).

Immune complex- mediated tissue damage appear to play a path genetic role intheextrahepatic manifestation of acute hepatitis B, the occasional prodromalserumsickness- like syndrome observed in acute hepatitis B appears to be related to thedeposition in tissue blood vessel walls of HBs Ag- Anti-HBs circulating immunecomplexes, lending to activation of the complement system and depressed serumcomplement levels. In patient with chronic hepatitis B, other types of immunecomplex disease may be seen. Glomerulonephritis with the nephritic syndrome isoccasionally observed; HBs Ag; immunoglobulins, and C3 deposition has beenfound in the glomerular basement membrane. While polyarteritisnodosadevelopsin considerably less than 1% of patient with chronic HBV infection, 20-30% ofpatient with ployarteritisnodoso have HBs Ag in serum. In these patient, theaffected small and medium size arterioles have been shown to contain HBs Ag,immunoglobulin, and complement components. Another extra hepatic manifestation of viral hepatitis, essential mixed cryoglobulnemia (EMC), was reported initially to be associated with hepatitis B(Kumar*et al.*, 2009, Ganem*and Alferd.*, 2004) and Jinlin*et al.*, 2005).

The disorder is characterized clinically by arthritis coetaneous vasculitis (palpablePurpura), and occasionally with Glomerulonephritis and serologically by the presence of circulating cyroprecipitable immune complexes of more than one immunoglobulin class (Ganem*and Alferd.*, 2004).

2.11 Laboratory diagnosis

2.11.1 Clinical chemistry tests

Clinical chemistry investigations are helpful in differentiating hepatocellular jaundice due to viral hepatitis from hemolytic jaundice and obstructive jaundice they include(Monica C., 2000).

a. Serum aminotransferase enzyme activities (ALT-AST) are increased in the pre-icteric stage.
b. Urobilinogen can be found in urine, and when there is jaundice.
c. Serum bilirubin levels are increased and bilirubin is present in the urine. Many viral hepatitis infections, however an icteric (without jaundice) but aminotransferase activity increased. Measurements of serum albumin can provideinformation on the severity of the hepatitis.

2.11.2 Serologicaltests

Hepatitis B is diagnosed from the results of specific HBV blood test (serology) that reflect the various component of the HBV.The diagnosis is made on the basis of the blood sample which used to demonstrate antibodies against hepatitis B, or hepatitis B components in the patient blood (El Mishad, 2007).

All patients with chronic infections have the viral component called HBs Ag. When both HBs Ag and HBe Ag are present, the infectiousness of the disease is at is highest and in

the long run, those patient are at increased risk of developing complications (El Mishad, 2007).

Hepatitis B surface antigen is the earliest indicator of hepatitis B infection. This Antigen may be present before symptoms of HBV infection are present. If this Ag level remains high for more than 6 months, this means the patient became a chronic carrier of HBV (El Mishad, 2007).

Hepatitis B surface Antibody (HBs Ab) usually appear about 4 weeks after hepatitis B surface Ag disappear. The presence of this antibody means that the infection is at the end of its active stage and the patient cannot pass the virus to others. Occasionally, your test may shows that you have both the HBs Ab and Ag antibodies indicating the presence of mutant strain of HBV. In this case, you are still contagious (El Mishad, 2007).

Hepatitis B envelope Ag (HBe Ag) is an HBV protein that is present during an active HBV infection. This is test determines how contagious the patient is testing for this antigen can also be used to monitor the effectiveness of treatment for HBV (El Mishad, 2007).

The hepatitis B virus DNA tests measure how much genetic material is present. A high level of HBV DNA means that the virus is multiplying in your body and you are very contagious. If you have chronic HBV, an elevated viral DNA level means you are an increased risk for liver damage and may want to consider treatment with antiviral medicine. Testing for HBV DNA is also used to monitor the effectiveness of treatment

for chronic HBV infection. HBV DNA testing is more sensitive test than HBe Ag for detecting HBV in the blood (Pommerville., 2004).

Other HBV tests are not done as often hepatitis B core Ab(HBcAb) is an antibody to the hepatitis B core antigen that appears about one month after an active HBV infection. It can be found in people who had an infection in the past and those with long-term (chronic) HBV. Some blood banks test for this antibody when screening donated blood for hepatitis B (Chempe*and Harvey*.,2008).

Anti-HBc is the first antibody appear and have a high titers of IgM, anti –HBcsuggest an acute and continuing viral replication. It persists for many months. IgManti-HBc may be the only serological indicator of recent HBV infection in a period when HBs Ag has disappear and anti –HBs is not detectable in the serum. Anti-HBe appears after the anti-HBc and is appearance relates to a decreased infectivity(Kumar*and Clark.*, 2009).

Enzyme linked immunosorbent assay (ELISA): The procedure involves attaching antibodies or antigens to a solid surface and combining (immunosorbent) the coated surfaces with the test material. An enzymes system then is linked to the complex, the remaining enzymes are washed away, andthe extend of enzyme activity is measured. This gives an indication that antigens or antibodies are present in the test material (Pommerville., 2004).

2.11.3 Polymerase chain reaction (PCR) test

It is based on the use of DNA fragment called the gene probe (39). Gene probe is relatively small, single stranded DNA segment that can hunt for complementary fragment of DNA (Mumtaz*et al.*, 2011).

To use a gene probe effectively, it is valuable to increase the DNA to be searched.The polymerase chain reaction (PCR)accomplishes this task (Pommerville., 2004).

2.11.4 Detection of HBV in tissue specimen

Because of its ability to generate high viremia and antigenaemia, HBV infection is usually easy to diagnose with the aid of serum sample. Liver biopsies are necessary to examine the degree of the inflammation, necrosis and fibrosis, and repeated biopsies are required to follow the progression of the disease or thesuccess of antiviral therapy. Because of the risk associated with biopsy, it should be done only if the clinical, biochemical and virological data suggested severe disease.Biopsy may be stained for HBs Ag, pre-s1 antigen, HBc Ag or HBV DNA(Topley*and Wilsns.*, 2011).

2.12 Treatment

Patient experiencing the acute symptoms of the hepatitis should receive symptomatic treatment. The most common complications from an acute infectionare dehydration and/or electrolyte imbalance from vomiting. This can corrected with fluid administration antiemetic may be employed (Kumar*and Clark.*, 2009). Treatment use for HBV infection are recombinant subcutaneous interferon Alfa (10Mu thrice weekly),oral

lamivudine(100mg once a day), oral adefovir (10mg once a day)and oral enticavir (0.5g once a day) has recently been approved. The main goal of treatment of chronic hepatitis B is to suppress HBV replication and to induce remission of the liver disease before development of cirrhosis and hepatocellular carcinoma (Souza*et al.,* 2004).

2.13Prevention and vaccination

Prevention depends on avoiding risk factors. These include not sharing needles and having safe sex, Standard safety precautions in laboratories and hospitals must be enforced strictly to avoid accidental needle punctures and contact with infected body fluids (Lung*and Fung*., 2008).

2.13.1Current vaccines

The currently used European HBV vaccines consist of the small envelope(s) protein and middle pre-s2 envelope (m) protein assembled into 22 nm particles.

Determinant and several subtype determinants. S vaccines are produced by processing of HBs Ag purified from plasma of HBV carrier and from yeast cells (*Saccharomyces cerevisiae*) expressing recombinant vaccine prepared by expression in CHO cells(Mumtaz*et al.,* 2011).

2.13.2Combined prophylaxis

It should be given to staff with accidental needle stick injury; all newborn babies of HBs-Ag –positive mothers; regular sexual partner of HBs Ag –positive patients, who have been found to be HBV-negative. For adult a dose of 500 IU of specific hepatitis B

immunoglobulin (HBIG) (200IU to new born) is given and the vaccine (I.M) is given at another site (Timbury., 1997).

12.13.3 Active immunization

This is with a recombinant yeast vaccine produced by insertion of a plasmid containing the gene of HBs Ag in to yeast. Dosage regimen. Three injections (at 0,1and 6 months) are given in to deltoid muscle ; this gives short-term protection in over 90% of patient – people who are over 50years of age or clinically ill and for immunocompromised (including those with HIV infection and AIDs) have a poor antibody response; more frequent and larger doses are required. Antibody levels should be measured at 7-9 months after the initial dose in all at-risk groups. There are few side-effects from vaccine, soreness at the site of injection may occurs, with very occasionally a fever, rash or a flu–like illness (Kumar*and Clark.,* 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1Study design

3.1.1 Type of study

This is a descriptive cross sectional study.

3.2.1Study area

The study was carried out in Uomdoreman Maternity HospitalKhartoum, Sudan.Practical part was of this study done in Disease preventive & screening Centre Laboratory in UAE.

3.3Study period

The study was carried out from October 2015 toDecember 2015.

3.4 Study population

Pregnantwomen attending Omdurman Maternity Hospital in Khartoum state.

3.5Study variable

Variable like age, gender, residence, martial state, tattooing, dental treatment, smoking, pregnancy, education and vaccination were considered.

3.6Sample size

A total of 90 samples whom agree to participate were enrolled pregnant women.

3.7Sample technique

It was convenient non probability random sample (quota).

3.8 Data collection

Data were collected using interviewing questionnaire covered general information which includeAge, , martial state, tattooing, dental treatment, smoking, education and vaccine (Appendix 1).

3.9 Collectionof blood specimens

Under sterile condition, 5ml of venous blood sample was withdrawn from each one using vacationer tubes. The serum was separated by centrifugation at 5000rpm for five minutes. Serum was store at -20^{o}C until used.

3.10 Detection of HBV

Sera samples were tested for the presence of HBs Ag using Enzyme LinkImmuno-Sorbent AssayELISA (Bio-Rad) (Monolisa HBs Ag ULTRA) (appendix 2) the procedure was doneaccording to the instructions of the manufacturer.

3.10.1 ELISA technique

3.10.1.1 Principles

An enzyme-immunoassay based on a sandwich principle was applied in this study. Microtiter well had been coated with monoclonal anti-HBs (antibody to HBs Ag), which constitute the solid-phase antibody. The test sample was incubated in each well; when HBs Ag present in the sample it bound to the solid-phase antibody. Subsequently guinea-pig anti-HBs, which have been labeled with the enzyme horseradish peroxidase (HRP), was added with positive reaction this labeled antibody becomes bound to any solid-phase antibody HBs Ag complex previously formed. Incubation with substrate produced a blue color in the test-well, which turns yellow when the reaction was stopped with sulphuric acid. When the sample did not contain HBs Ag, the labeled antibody cannot be bound specifically and only a low background color developed.

3.10.1.2 Assay procedure

The reagent and sera were allowed to reach room temperature. 100 uL of each control (Non-Reactive Control, and Reactive Control) and serum were added to the appropriate microtiter Plate well, and then 50ul of HRP-conjugate was transferred to each well except blank and mixed well and incubated for 60 minutes at 37^{o}C. The Microtiter Plate was washed 4 times and soaked for second 20-30seconds with diluted wash buffer. 100 uL of substrate was added into each and incubated for 30 minutes at 37^{o}C.This distribution can be visually controlled: There is a clear different of coloration between empty well and well with the pink substrate solution. This distribution can also be controlled automatically riding at 490 nm (optional).

100 uL stop solution was addedthis distribution can be visuallycontrolled. Thesubstrateinitiallypink become uncolored for the non-reactive sample wells andturn blue to yellow for the positive well (R3).

Reading of the optical densities at 450/620- 700 nm and interpretation of the result, wereinterpreted.

Reader

Calculate the mean measured absorbance value for negative control (A1, B1, G1, and D1).

Calculated cut-off: mean R3 + 0.050.

Positive test is <R3+ 0.050.

Negative test>R3+0.050.

3.11 Data analysis

Processing and analysis of data were carried out by means of the statistical package for the social sciences (SPSS). A descriptive statistic frequency was used to assess the risk , Cross tabulation (chi-squire) was used to compare the variable with positive result.

3.12 Ethical consideration

Each individual invited to participate in the study was informed about the purpose of study before data collection. The study was approval by Ethicalcommittee, College of Medical Laboratory Science, Sudan University of Science and Technology.

CHAPTER FOUR

RESULTS

4.1 Results

Ninety pregnant womenwere participated in this study. All participants fall in age ranged from 15 to 45years old.The mean age was 24 years; Most of participants (62.2 %) fall in age ranged 26-35 (%). According to geographical residence, the majority 53 (58.9 %) from Khartoum, Bahri 20 (21.8%), Omdurman 9 (9.8%) and out of Khartoum 8 (9.5%). 59(65.6%) not vaccinated will 31 vaccinated (34.4%). 77 (85.6%) were house wifewhile 12(14.4%)employee (Table 1).

The hepatitis B surface antigen were founded in 5(5.6%) of study population. This rest (85) were negative for Hepatitis B viruswhile 5 were positive for Hepatitis B virus Table (2).Most of the infected women were in age (26-35).

Table 2 showed statistical relation of HBsAgsero-diagnosis and its risk factors among pregnant women using ELISA technique.

The results indicated significant relations (P value < 0.05) between HBsAg infection HBsAg and dental treating and blood transfusion. While it has insignificant association with vaccine, residence, age, occupationand gestational age Table (2)

Table 1.Frequency of risk factors of HepatitisB virusinfection among study population

Risk factor	Result	Count Frequency	Percent
Blood transfusion	Yes	9	10.0
	No	81	90.0
Dentistry	Yes	9	10.0
	No	81	90.0
Residence	Khartoum	53	58.9
	Bahry	20	22.2
	Umdurman	9	10.0
	Out of Khartoum	8	8.9
Occupation	Housewife	77	85.6
	Employee	12	13.3
Vaccine	Yes	31	34.4
	No	59	65.6
Gestational age	1st trimester	18	20.0
	2nd trimester	22	24.4
	3rd trimester	49	54.4
Age groups	15-25	22	24.4
	26-35	56	62.2
	36-45	12	13.3

Table 2. Statistical relation of sero-positiveHBs Ag using ELIA technique and its risk factors among pregnant women

Risk factor	Result	HBsAg Positive	HBsAg negative	p.value
		Count	Count	
Blood transfusion	Yes	5	4	0.000
	No	0	81	
Dentistry	Yes	5	4	0.000
	No	0	81	
Residence	Khartoum	5	48	0.106
	Bahry	0	20	
	Omdurman	0	9	
	Out of Khartoum	0	8	
Occupation	housewife	3	74	0.075
	Employee	2	10	
Vaccine	Yes	0	59	0.159
	No	5	26	
Gestational age	1st trimester	1	17	0.738
	2nd trimester	2	20	
	3rd trimester	2	47	
Age groups	15-25	1	21	0.766
	26-35	3	53	
	36-45	1	11	

- Spearman`s correlation was used to calculate P value.
- P value <0.05 consider significant.

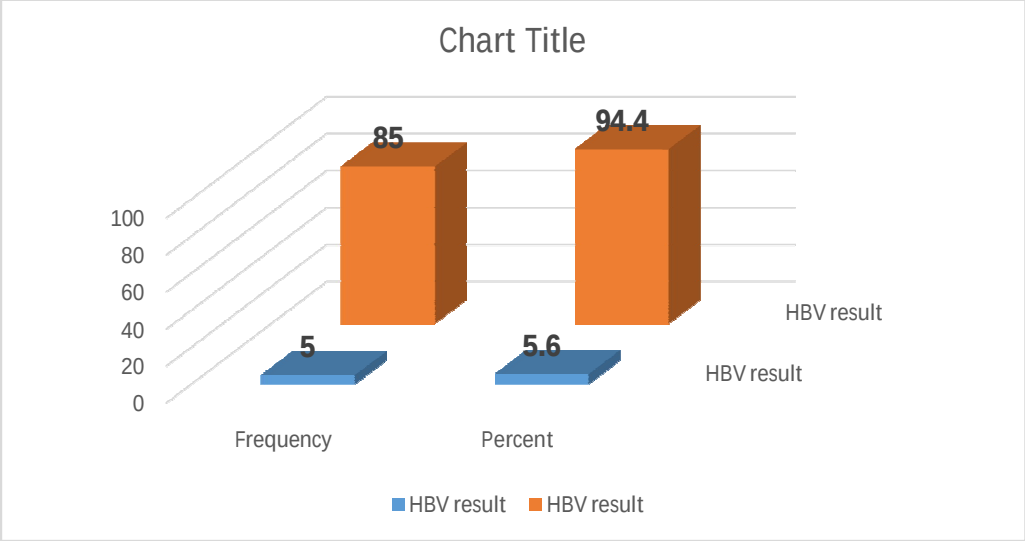

Fig 1. Frequency of the HBsAg result among pregnant women.

CHAPTER FIVE

DISCUSSION

5.1 Discussion

Hepatitis B is a disease of significant public health impact. Prevention strategies are important for the effective control of this infection, including universal vaccination and the use of immunoglobulin in certain situations. For this purpose, data about prevalence rates are needed, especially in populations at risk for disease transmission, such as pregnant women.

In this study the frequency of HBs Ag among pregnant women was found similar to that reported carried out in Brazil (Souza*et al* ., 2012)relatively low compared to study carried out by (Abou *et al*.,2009 and Ding Y *et al*., 2013). The difference of frequency of HBs Ag among the same subject might be owing to diverse of life, influence of risk factorand to the method used to assess the occurrence of hepatitis infection.

Generally, results of this study were considerably lower than that reported at Central Sudan(6.8%) (Mudawi ., 2008) and at South Sudan (26.4%), (Abou *et al*., 2009) reported 6.25% sero-positive among blood donors in Nyala, south Darfur, however Sudan is considered as endemic area of HBV infection, (Elsheik *et al*.,2007)reported sero-prevalence of 5.6% among antenatal care attendants in central Sudan.

The present study revealed that most candidate had not received the vaccine, even so (Hungand his colleaguesreported moderate response to HBV vaccine (51.7%) among street involving youths Aged 15-24 years old (Gilbert*et al*., 2010).

The present results showed significant statistical relation between HBs Ag and blood transfusion, and dental treatment this result similar to study carried out by Souza *et al.*, 2004).

Agegroup, residence, occupation, gestational age and vaccine did not contribute significantly to increase HBs Ag in this study. Similarly, (Fathimoghaddam *et al*., 2011) and Khamduang*et al*,(2013), reported only age as risk factor affecting HBV infection among Mashhad population.

5.2Conclusion

1. 5 (5.6 %) of the study population were sero-positive for HBs Ag.
2. The results showed significant association of HBs Ag and dental treating, blood transfusion.
3. The study indicated insignificant (P<0.05) association between HBV infection and other factors, including residence, age, occupation and gestational age.

5.3Recommendations

1. HBV vaccine should be adjusted to cover all pregnant women.
2. Establishment of health education program as well academic education for increased awareness about hepatitis.
3. Hepatitis should be diagnosed as routine investigation among pregnant women.
4. Further studies with large number of participants are required to validate the result of this study.

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Appendices

Questionnaire on Study of Sero-diagnosis of Hepatitis B Surface Antigen among Pregnant Women in Khartoum State

Data collection Sheet

General data

1. Name……..

2. Gender

Male ( ) Female ( )

3. Age…….

4. Education level

Primary ( ) secondary ( ) university ( )

5. Blood transfusion

Yes ( ) No ( )

6. Dental treatment

Yes ( ) No ( )

7. Tattooing

Yes ( ) No ( )

8. Vaccine

Yes ( ) No ( )