بسم الله الرحمن الرحيم

Sudan University of Science and Technology

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Frequency of Recent Hepatitis E Virus Infection among Pregnant Women in Red Sea State

تكرار العدوى الحديثة لفيروس إلتهاب الكبد (هـ) لدى الحوامل في ولاية البحر الأحمر

A Dissertation Submitted in Partial Fulfillment of the Requirements of M.Sc. in Medical Laboratory Science (Microbiology)

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الآيــة

قال تعالى (**وَلَا تَقْفُ مَا لَيْسَ لَكَ بِهِ عِلْمٌ إِنَّ السَّمْعَ وَالْبَصَرَ وَالْفُؤَادَ كُلُّ أُولَئِكَ كَانَ عَنْهُ مَسْئُولًا (36) وَلَا تَمْشِ فِي الْأَرْضِ مَرَحًا إِنَّكَ لَنْ تَخْرِقَ الْأَرْضَ وَلَنْ تَبْلُغَ الْجِبَالَ طُولًا (37)**)

صدق الله العظيم

سورة الاسراء : الآيات (36-37)

DEDICATION

To my

Father,

Mother,

Sisters, and

Leen.

ACKNOWLEDGEMENT

First of all, thanks to ALMIGHTY ALLAH for giving me patience and support to complete this work.

I would like to express my gratitude to my supervisor **Prof. Humodi Ahmed Saeed for** his guidance through all stages of this study.

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ABSTRACT

Hepatitis E virus (HEV) is an important enteric human pathogen worldwide. It can cause sporadic cases as well as large epidemic of acute hepatitis. Many studies proved that hepatitis infection during pregnancy and in the third trimester especially with genotype 1, is associated with more severe infection and might lead to fulminant hepatic failure and maternal death.

The current study aimed to determine the frequency of HEV among pregnant women attending in Port Sudan Maternity Hospital in Red Sea State during the period from April to July 2017.

A total of 93 pregnant women were selected to participate in this study. Socio-demographic data including age, gestational age, history of previous abortion, and history of jaundice were collected by structured questionnaire.

Five milliliter of blood specimen was collected from each participant on EDTA container. Plasma was separated by centrifugation at 3000 rpm for 5 minutes. The plasma were transported to the Research Laboratory in ice bag then were stored at -70℃ until used. Analyze of all specimens was carried out for anti HEV IgM by ELISA technique.

The results of socio-demographic data revealed that age range of pregnant women was between 15 and 45 years. The majority 48(51.6%) of study population were at age between 26-35 years. Regarding the gestational age, 19(20%) were at first trimester, 37(40%) were at second trimester and 37(40%) were at third trimester, the history of abortion was found in 12 (13%), While the history of jaundice was found in 18 (19%).Investigation of plasma by ELISA technique revealed that all specimens were non-reactive and were considered free of HEV.

Further studies with large sample size and advanced technique are required to validate the results of the present study.

المستخلص

فيروس التهاب الكبد (هـ) هو مرض معدي يصيب الإنسان منتشر في أنحاء العالم . هذا الفيروس يمكن أن يسبب حالات متفرقة فضلا عن أوبئة واسعة النطاق من التهاب الكبد الحاد.

أثبتت العديد من الدراسات أن الإصابة بالتهاب الكبد (هـ) أثناء الحمل خصوصا في الثلث الأخير من الحمل خاصة مع النمط الجيني 1 ، ويرتبط مع عدوي أكثر حده ويمكن أن يؤدي إلي فشل كبدي مداهم ووفاة الأمهات

الدراسة الحالية هي دراسة تهدف لتحديد انتشار الإصابة بالتهاب الكبد الفيروسي (هـ) بين الناس الحوامل التي يترددن في مستشفي بور تسودان في ولاية البحر الأحمر في الفترة من ابريل حتى يوليو في العام 2017

تم اختيار 93 امرأة حامل للمشاركة في هذه الدراسة،بعض البيانات الديمقراطية وتضمنت العمر ،مدة الحمل ،حالات الإجهاض السابق ‘وظهور اليرقان عن طريق هيكل الاستبيان .

تم جمع 5 ملميترات من الدم من كل مشارك ومن ثم فصل البلازما بواسطة جهاز الطرد المركزي عند3000 دورة لمدة خمس دقائق وتم نقل البلازما في ثلج وتم تخزينها في −70ومن ثم فحصها عن مدي إنتشار الأجسام المضادة من نمط (الغلوبولين المناعي م) ،بواسطة طريقة الإليزا.

كشفت نتائج البيانات الاجتماعية الديمقراطية ان الفئة العمرية من النساء الحوامل كان مابين 15و45 سنة وكانت الغالبية 48(51.6%) من سكان الدراسة في سن ما بين 26−35 سنة، فيما يتعلق بعمر الحمل19(20%) في الاشهر الثلاثة الاولي. و37(40%) كانو في الثلث الثانية، 37(40%) في الثلث الثالث من الحمل، ووجد تاريخ الإجهاض 12(13%)، تم العثور علي تاريخ اليرقان 18(19%).

هنالك حاجه إلي مزيد من الدراسات مع زيادة حجم العينة ممطلوب تقنية متقدمة للتحقق من صحة هذه الدراسة لفهم افضل لالتهاب الكبد الفيروسي (هـ).

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LIST OF ABBREVIATIONS

HEV: Hepatitis E Virus

AHE: Acute Hepatitis E

ALF: Acute Liver Failure

ANC: Attending antenatal Clinic

EDTA: Ethylediaminetetracetic acid

ELISA: Enzyme Linked Immunosorbent Assay

FDA: Food and Drug Administration

H_2O_2: Hydrogen peroxide

HAV: Hepatitis A Virus

HIV: Human Immunodeficiency Virus

ICTV: International Committee on Taxonomy of Virus

IFN-α:Interferon α

IgA: Immunoglobulin A

IgG: Immunoglobulin G

IgM: Immunoglobulin M

ORF: Open Reading Frames

RBV: ribavirin

RNA: Ribonucleic acid

RT-PCR: Real Time-Polymerase Chain Reaction

SVR: Sustained Virological Response

TMB: Tetramethylebeenzedin

U.S: United States

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

CHAPTER ONE

INTRODUCTION AND OBJECTIVE

1.1 Introduction

Hepatitis E virus (HEV) is a positive strand RNA virus with three open reading frames which is transmitted predominantly through the fecal contamination of water and food. It is the most common cause of acute liver failure in endemic areas. Pregnant women especially from the Indian subcontinent and Africa are at increased risk of contracting acute HEV infection as well as developing severe complications including ALF. Transmission of HEV occurs from mother to unborn child. Both maternal and fetal complications may occur, including abortion, fetal demise, preterm labor and maternal or neonatal death (Adjei *et al*., 2009).

It is also recognized as an important cause of sporadic and epidemic of acute viral hepatitis in many developing countries in Asia, Africa and Central America (Teshale and Hu, 2011). The prevalence of HEV infection is between 7.2% and 24.5percent in developing countries with poor hygiene conditions, while the rate ranges around 0–3% in developed countries (Cevrioglu *et al*., 2004). Although HEV infection leads to self-limited inflammatory liver disease in immunecompetent patients, such infection can lead to acute hepatitis E associated with significant morbidity and mortality particularly in pregnant women where it becomes severe and with high mortality rate (Wedemeyer and Pischke , 2011).

Hepatitis E virus (HEV) is an enterically transmitted pathogen and is responsible for recent large-scale epidemics of hepatitis around the world, as reported recently in Uganda, where more than 7500 cases were registered in 1 year. HEV induces self-limiting or acute hepatitis, and the severity can varied from no symptoms to fulminating infection (Emerson and Purcell, 2008). HEV infections have not been

known to become chronic (Emerson and Purcell., 2008) however, recently, persistent HEV infection, with chronic hepatitis and cirrhosis, has been reported in patients with reduced immune surveillance induced by chemotherapy or post-transplant immune suppression (Gerolami *et al*.,2008). The average mortality rate from HEV infection is 1–4%, principally among adolescents and young adults, but it is still not clear that the severity is age dependent. For unknown reasons, the mortality rate is higher among pregnant women, especially during the third trimester (Purcell and Emerson. 2008). In Sudan, a case fatality ratio of 17.8% was found in an outbreak in Darfur, with a ratio of 31.1% among pregnant women (Boccia *et al*.,2006).

Few data are available on the circulation of HEV in central Africa. In 1995, no anti-HEV IgG was found in samples collected in Libreville, the capital of Gabon (Richard *et al.,* 1995) but the study was based on a small sample and did not reflect the actual situation in the country. Furthermore, the laboratory techniques for HEV detection have advanced considerably since the time of that study. The aim of the study reported here was to evaluate the prevalence of anti-HEV IgG in samples collected from pregnant women living in the five main cities of Gabon. We also compared the HEV prevalence in rural and urban areas in the region with the highest seroprevalence

1.2 Rationale

Hepatitis E virus (HEV) mostly causes a self-limited disease in developing countries, but the nature of disease is more severe in pregnant women, due to hormonal changes (estrogen and progesterone) during pregnancy. The infection during pregnancy associated with poor fetal outcomes including abortion, premature delivery, and stillbirth (Tabatabi *et al*., 2014).

The average mortality rate from HEV infection is 1–4%, principally among adolescents and young adults, but it is still not clear that the severity is age-dependent. For unknown reasons, the mortality rate is higher among pregnant women, especially during the third trimester (Purcell and Emerson.2008). In Sudan, a case: fatality ratio of 17.8% was found in an outbreak in Darfur, with a ratio of 31.1% among pregnant women (Boccia *et al*.,2006).

1.3 Objectives

1.3.1General Objective

To investigate HEV among pregnant women in Red Sea State, using ELISA.

1.3.2 Specific Objectives

(I) To detect HEV IgM antibody.

(II) To determine the frequency of HEV among pregnant women

(III) To detect relation between the presence of HEV antibodies and gestational stages.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1. Hepatitis E Virus

The HEV first retrospectively confirmed outbreak of hepatitis E occurred in 1955-1956 in New Delhi, India and resulted in more than 29000 symptomatic jaundiced persons. Since that time, many large outbreaks have occurred in Asia, Africa and Mexico. In addition, sporadic hepatitis E outbreaks commonly occur in developing countries of Asia and Africa as well as in industrialized countries. Although there is a distinct epidemiologic picture of HEV infection in North America, Europe, and Japan, this review article will summarize the current epidemiology of HEV infection in the developing world HEV.

The discovery of HEV followed the development of serological tests for hepatitis A virus (HAV) (Coursaget *et al*., 1998).

2.1.1. Classification of hepatitis E virus

HEV was previously classified as the sole member of the *Hepevirus* genus of the *Hepeviridae* family, however with the identification of novel HEV-like viruses in a variety of animals, an adjustment of the taxonomic classification was recently ratified by the International Committee on Taxonomy of Viruses (ICTV). The *Hepeviridae* family is now subdivided into two genera: the *Orthohepevirus* that comprise all mammalian and avian HEV isolates and the *Piscihepevirus* that comprises the cutthroat trout virus. The *Orthohepevirus* is further subdivided into four species: *Orthohepevirus A* (comprising human, pig, wild boar, deer, mongoose, rabbit, and camel isolates); *Orthohepevirus B* (comprising isolates from chicken); *Orthohepevirus C* (comprising isolates from rat, greater bandicoot, Asian musk shrew, ferret, and mink); and *Orthohepevirus D* (isolates from bat). Whereas the ICTV does not provide a classification below the species level (Purdy *et al.*, 2014).

Smith *et al.* proposed to subdivide the *Orthohepevirus A* species into seven genotypes: Genotypes 1 and 2 only infect humans, mainly in endemic areas such as Asia, Africa, and Mexico (Holla *et al*., 2013).

Genotypes 3 and 4 include zoonotic strains that can infect both pigs and humans, but also include strains that have been isolated from rabbits, deer, and mongoose. Genotype 3 viruses are responsible for most auto chthonous HEV infections in Europe (Sayed *et al*., 2015).

Genotype 4 is mainly found in Asia, where it causes sporadic cases of acute hepatitis E (AHE) in humans. Strains isolated from wild boar are classified in genotype 5 and 6, whereas isolates from camel are classified into genotype 7 (Lu *et al*., 2006).

2.1.2. Morphology

Hepatitis E virus virions are small, non-enveloped, 32-34 nm diameter particles with icosahedral symmetry. The viral genome, approximately 7.5 kb in length, is a single-stranded, positive-sense, polyadenylated RNA molecule that contains short 5′ and 3′ non-coding regions of 27 and 68 nucleotides, respectively contains three overlapping open reading frames (ORF) (Lu *et al*., 2006).

2.1.3. Genome and proteins

The organization of the HEV genome is substantially different from other viruses and it has its own family, the hepeviridae, genus hepevirus, species HEV (Schlauder and Mushahwar., 2001).

The HEV genome is arranged in three overlapping open reading frames (ORF). The three coding frames are used to express different proteins. ORF1 encodes a polyprotein of about 1690 amino acids that undergoes post-translational cleavage into multiple nonstructural proteins required for virus replication, including a methyl transferees, a putative papain-like cysteine protease, an RNA helicase and an RNA-dependent RNA

polymerase. ORF2 does not overlap with ORF1; it is located at the 3'-end of the genome and encodes the principal structural protein, the capsid protein of 660 amino acids. ORF3 begins with the last nucleotide of ORF1; it overlaps with ORF2 and encodes for a small immunogenic123 amino acid phosphoprotein which associates with the cytoskeleton, suggesting its possible role in the assembly of virus particles (Bradley, 1995).

The organization of the ORF differs slightly according to genotype however the function remains the same. Compared to the HAV, the HEV is less resistant to environmental conditions such as temperature (Lu *et al.*, 2006).

2.1.4. Transmission

HEV can spread by four different transmission modes: fecal-oral, food-borne, blood-borne, and vertical transmission. The fecal-oral route, mainly by ingestion of contaminated water, is the most common mode of transmission of HEV infection globally and is responsible for the majority of HEV outbreaks (Yugo and Meng., 2013). Food-borne HEV transmission is achieved by consumption of raw or undercooked meat from infected animals, typically pigs and wild boar, or contaminated seafood (Sayed *et al*., 2015).

HEV RNA has also been detected on leafy green vegetables and on field-grown strawberries, probably caused by the use of contaminated irrigation water and/or contaminated manure or compost (Kokkinos *et al*., 2012).

Vertical or perinatal transmission from mother to child has also been observed in developing countries (Krain *et al*., 2014).

Blood transfusion–associated HEV transmission has not only been documented in several European countries, such as Great Britain, Germany, and France but also in Canada and Japan (Andonov *et al*., 2014).

2.1.5. Clinical Presentation

The incubation period of HEV infection ranges from 15 d to 60 d (mean 40 d). Research among non-human primates showed a direct association between infective dose and severity of disease with an inverse relation to the incubation period (Tsarev *et al*., 1993)

HEV causes a range of clinical manifestations including asymptomatic infection, unapparent infection, and icteric hepatitis. The clinical presentation of acute hepatitis E is indistinguishable from other acute viral hepatitis. Acute disease with abrupt onset of non specific symptoms followed by right upper quadrant pain, jaundice, anorexia, malaise nausea and vomiting. Asymptomatic infections occur more often among children than adults. In a study by Khuroo., *et al* the symptomatic to asymptomatic ratio for children was 1:12 while for adults it was only 1:3. The symptomatic hepatitis E attack rate during an outbreak can reach up to 15%. The attack rate is always higher among adults even in countries where HEV epidemics have occurred repeatedly. Furthermore, the attack rate varies by gender. In many epidemics men were found to have higher attack rates. However, in a recent HEV outbreak in Uganda, women were more likely to have symptomatic hepatitis E than men and this difference was significant for women aged 15-45 years. The symptomatic to asymptomatic ratio for children was 1:12 while for adults it was only 1:3. The symptomatic hepatitis E attack rate during an outbreak can reach up to 15% (Khuroo *et al*., 1994).

The attack rate is always higher among adults even in countries where HEV epidemics have occurred repeatedly. Furthermore, the attack rate varies by gender, in many epidemics men were found to have higher attack rates. However, in a recent HEV outbreak in Uganda, women were more likely to have symptomatic hepatitis E than men and this difference was significant for women aged 15-45 years (Teshale *et al*., 2010).

2.1.6. Pathogenesis

Several elements of pathogenesis can be outlined on the basis of data from human patients and those from experimentally infected animals. The incubation period in human volunteers after oral exposure is 4–5 weeks, but the route and mechanism by which the virus reaches the liver from the intestinal tract remain unknown. Hepatitis E virus can be detected in stools beginning approximately 1 week before the onset of illness and persists for as long as 2 weeks thereafter (Krawczynski *et al*., 1996).

Hepatitis E virus-RNA can be detected in faeces of most patients with acute hepatitis E by RT-PCR for approximately 2 weeks; in some cases, RT-PCR has yielded positive results for as long as 52 days after onset The HEV-RNA has regularly been found in serum by RT-PCR in virtually all patients in the first 2 weeks after the onset of illness; prolonged periods of HEV-RNA positivity in serum ranging from 4 to 16 weeks have also been reported (Nanda *et al*., 1995).

2.1.7. Host immune response

Viremia in bile and serum and shedding of HEV in feaces reach their peak during the incubation period and keep constant level in the acute phase of the disease, at the same time HEV antigen can be detected in the liver, although the period of infectivity after acute infection has not been determined, virus excretion in faeces has been demonstrated up to 14 days after onset of jaundice then disappears during the recovery phase (Mirazo *et al*., 2014).

Antibodies to HEV (IgM and IgG) develop at the time symptoms occur ,usually before the development of jaundice, IgM anti-HEV precedes the IgG anti-HEV by few days and viremia may persist after appearance of serum antibody(Candido *et al*., 2012)

IgM anti HEV titers decline rapidly during early convalescence 48 while IgG anti HEV have been shown to persist for long periods of time (more14 yrs) and provide protection against subsequent infection (Bendall *et al*., 2010).

2.1.8. HEV in pregnant women

The outcome of disease is worse in pregnant women, there is no evidence to suggest that they are more susceptible to infection or are at higher risk of infection. HEV infection in pregnant women is typically severe during the third trimester of pregnancy. Mortality rates among pregnant women in the third trimester range from 10%-25%. To date, it is not clear what the disproportionately high mortality among pregnant women is due to the causes of death include fulminant liver failure and obstetric complications including excessive bleeding (Hussaini *et al*., 1997).

In contrast, the high mortality observed in many Asian and African countries was not observed among HEV infected pregnant women from Egypt. No deaths among more than 2000 pregnant women with serological markers for infection (Bacciad *et al.*, 2006).

2.1.9. HEV in developing countries

HEV infection is a major public health problem in many developing countries. The first documented outbreak of hepatitis E in India resulted in 29,300 cases of jaundice. Other notable large outbreaks, resulting in significant morbidity, include outbreaks in India and China that resulted in 79,000 cases and 119,000 cases respectively (Zhang *et al.*, 1991). Since genotyping of HEV strains became common in the mid-1990s, hepatitis outbreaks in developing countries have been caused primarily by HEV genotype 1, with outbreaks in Mexico and western Africa caused by genotype 2 (Maila *et al*., 1995).

Sporadic cases in Asia caused by genotype 4 in countries with suboptimal sanitary conditions, HEV is the single most important cause of sporadic and epidemic hepatitis. In susceptible populations, high attack rates have been observed (Teshale *et al*., 2010). Case-fatality rates in epidemics range from 0.2% to 4%, but pregnant women, especially during the third trimester, may have a case-fatality rate of 10%–25% (Khuroo *et al*., 1995).

In areas of endemicity, HEV is the most common cause of hepatitis during pregnancy. In studies involving pregnant women, HEV accounted for 37% of cases of acute viral hepatitis and 81% of cases of fulminant hepatitis, with more than one-fourth of affected women having obstetric complications, such as premature rupture of membranes and intrauterine growth restriction. It is unknown why HEV causes severe disease in pregnant women. Among pregnant women who are exposed to HEV during the third trimester, death is usually due to fulminant hepatitis or obstetric complications (Tsega *et al*., 1993). Vertical transmission with consequent morbidity and mortality of infants is also common with third trimester hepatitis E, however, in Egypt where prevalence of anti-HEV antibody in the rural population is high, compared with in other countries severe cases of hepatitis E have never been reported (Stoszek *et al*., 2006).

In countries where outbreaks occur, HEV genotype 1 infection has been considered a waterborne infection transmitted through drinking of fecally contaminated water (Tsega *et al*., 1993).

2.1.10. Epidemiology

In developing countries, hepatitis E occurs both sporadically and as epidemic disease, affects a large proportion of the population, and is largely due to genotype 1 (with genotype 2 accounting for cases in Mexico and parts of Africa, Published rates of anti-HEV antibody among adults in these areas range from 30 to 80%. In Bangladesh, the incidence of HEV infection was studied prospectively in cohorts of both the general population and pregnant women, who are especially prone to fulminant hepatitis E (Labrique *et al.*, 2010).

In the population at large, the incidence of HEV infection was 6.4% among approximately 1200 people across all ages. In two cohorts of pregnant women, the annual incidence of HEV infection was 4.6% and 5.6%. Assessment of the women's micronutrient status and serum cytokine levels suggested that nutritional and

immunologic features played a role in the susceptibility to severe infection; these findings indicate potential inroads in efforts to reduce morbidity and mortality associated with HEV infection (Labrique *et al.,* 2010).

Cases of acute hepatitis E account for a large proportion of cases of acute liver disease in developing countries, with smaller (although unknown) proportions in Europe and the United States.36 In developed countries, individual cases and small outbreaks have been linked to exposure to pigs and consumption of undercooked pork or wild game (Wichmann *et al.,* 2008).

Testing of samples of pig liver and sausage from commercial groceries in Europe and the United States identified HEV RNA in a high percentage of samples. Furthermore, laboratory analyses showed the presence of infectious HEV in rare and medium-rare meat. Case reports have also linked hepatitis E to consumption of shellfish 38 and to blood transfusions (Matsubayashi *et al.*, 2008).

The overall rate of these risk factors among unselected patients is low. Thus, most patients with autochthonous hepatitis E report no specific risk factors, such as exposure to pigs or consumption of undercooked pork or sausage. Furthermore, secondary spread is rare, if it occurs at all. Only small numbers of cases have been reported in the United States, many of which were misdiagnosed as drug-induced liver injury (Davern *et al*., 2011).

2.1.11. Chronic Hepatitis E

HEV was initially thought to resemble hepatitis A virus, causing acute, self-limited infections only; thus, it came as a surprise when cases of chronic hepatitis E were described. Chronic infection has been identified almost exclusively among immunocompromised persons, including organ-transplant recipients, patients receiving cancer chemotherapy, and HIV-infected persons (Dalton et *al.*, 2009).

HEV RNA has been detected in moderate-to-high levels in serum and stool and has persisted for years. Serum aminotransferase levels have also been abnormal, and some patients have had progressive liver disease with fibrosis or cirrhosis. Chronic Hepatitis E and Response to Antiviral Therapy. Chronic HEV infection may also occur in adults without apparent immunodeficiency, although such cases are rare (Gonzalez *et al.*, 2011). The source of infection is often unknown, but in a minority of cases, blood transfusions or the organ itself have appeared to be the source. The unreliability of antibody tests and the need for direct molecular assays to detect HEV infection pose diagnostic challenges in this population (Gonzalez *et al.*, 2011).

2.1.12 . Diagnostic Assays

As in other forms of viral hepatitis, viremia arises during the incubation period of hepatitis E, and antibodies (both IgG and IgM) appear at the time of clinical onset, just preceding elevations in serum aminotransferase levels and symptoms Course of Acute HEV Infection. Recovery is marked by viral clearance, an increase in IgG titers, and a decrease in IgM levels. HEV is also present in stool, usually during the incubation period, throughout active infection, and in the initial part of the recovery period. The duration of viral shedding is variable, as is the presence of antibodies. IgM anti-HEV antibody remains detectable for only 3 to 12 months, whereas IgG anti-HEV antibody persists for years, if not for life (Chandra *et al.*, 2010).

Tests for anti-HEV antibody (including IgG- and IgM-specific assays) are available commercially, but none have formal Food and Drug Administration (FDA) approval. Unfortunately, the sensitivity and specificity of available assays vary widely; this may account for the discrepancies among published rates of anti-HEV antibody in various populations. In studies from the United Kingdom, the prevalence of anti-HEV antibody in a blood-donor population was 3.6% with the use of one commercial assay and 16.2% with the use of another. Similarly, serum samples obtained from patients with acute HEV infection (documented on the basis of detectable HEV RNA during the acute phase) were

reactive in 44% of patients with one assay and in 98% with the other (Bendall *et al*, 2010).

Until assays receive FDA approval, physicians must depend on whatever test is locally available. Patients with unexplained acute or chronic hepatitis should be tested for IgM anti-HEV antibody, with a positive result suggesting ongoing infection. Tests for HEV RNA in serum and stool are confirmatory but are currently still experimental. Serologic and virologic testing is available on a limited basis from the Centers for Disease Control and Prevention (Bendall *et al.*, 2010).

2.1.13. Treatment

Immune competent persons are usually able to spontaneously clear an acute HEV infection. Hence, most AHE infections do not require treatment. However, pregnant women, people with pre-existing liver disease, and immune suppressed transplant patients may require therapy (Lens *et al.*, 2015).

Reduction of immunosuppressive therapy in transplant recipients leads to HEV clearance in approximately 30% of HEV-infected transplant recipients. If this strategy is not tolerated or if it does not achieve the desirable results, either treatment with pegylated interferon alpha (Peg-IFN-α) or ribavirin (RBV) could be started. In principle, antiviral therapy should be started if viral clearance is not attained within 3-6 months Peg-IFN-α was first described for treatment of chronic HEV in 2010 (Kamar *et al.*, 2010).

Although a 3- to 12-month therapy usually, but not always, resulted in a sustained virological response (SVR), Peg-IFN-α therapy remains tedious in organ transplant patients because of the severe side effects and potential graft rejection. Especially, heart and kidney transplant patients are at high risk of graft rejection. RBV therapy should be the first-choice antiviral treatment in transplant patients (Kamar *et al.*, 2010).

RBV inhibits HEV replication through depletion of cellular guano sine triphosphate pools and is moderately synergistic with interferon (IFN) therapy. RBV therapy for at least 3 months usually results in clearance of infection. Although RBV therapy seems to be very effective, up to 38% of treated patients do not show an SVR or even relapse during therapy. Usually, this is linked to RBV dose reductions because of severe anemia, but also a mutation in the viral polymerase (G1634R), which results in more-efficient replication, was linked to partial or non response because RBV is contraindicated during pregnancy owing to its teratogenicity and also peg-IFN-α cannot be used in this setting, novel antiviral strategies are highly needed (Debing and Neyts, 2014).

2.2. Previous Studies

A study in Ethiopia was aimed to determine the seroprevalance and risk factor of HEV infection among pregnant women attending antenatal clinic (ANC) in Addis Ababa, Ethiopia from April 2014-January 2015. This study found a high seroprevalance rate of anti HEV IgM among pregnant women 31.6% (Meseret. 2015).

Another study was conducted in India to determine seroprevalance of HEV in pregnant women attending Iman Khomeini general hospital in Ahvaz, India in the period from january2010 to January 2011. Overall, 2.26% cases were positive for anti HEV IgM among pregnant women (Resti *et al*., 2014).

Also a study was conducted in the Obstetrics and Gynecology Outpatient clinic of KBTH, Accra, Ghana to evaluate the prevalence of anti-HEV IgM among pregnant women seen between the months of January and May, 2008.This study indicate that Ghana is an endemic area for hepatitis E, with very high overall prevalence rate of HEV IgM28.66% (Adje *et al*., 2009).

2.3. Prevention of HEV Infection

Improved personal hygiene and sanitation has led to a marked reduction in the incidence of HEV infection, mainly in developing countries. However, pigs

represent the major animal reservoir of HEV in industrialized countries and are most likely the main cause of infection. Surveys of European and U.S. pig farms indicate that a large proportion of herds have evidence of recent or ongoing HEV infection (Meng, 2013). Although detection and isolation of infected pigs and pork meat could represent the first step in the prevention of HEV spread, this strategy is most likely practically and economically not achievable. However, adequate cooking of pork meat by heating to an internal temperature of 71°C for, depending on the food type and fat content, 1-20 minutes completely inactivates HEV and consequently reduces the possibility of infection by ingestion (Brassard *et al.*, 2012).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

This is a descriptive cross-sectional study.

3.1.1. Study area

The study was conducted in Port Sudan Maternity Hospital in Red Sea State.

3.1.2. Study population

Pregnant women visited Port Sudan Maternity Hospital in Red Sea state.

3.1.3. Sample size

In this study 93 pregnant women were enrolled.

3.1.4. Study duration

The study was conducted during the period from April to July 2017.

3.2. Data collection techniques and tools

Data were collected by interviewing each participant using structured questionnaire (Appendix1), The data including age, gestational age, history of previous abortion, and history of jaundice.

3.3. Data processing

All data were coded then computerized and analyzed using Statistical package for social science (SPSS).

3.4. Ethical consideration

The ethical clearance was obtained from the Ethical Committee Board of Sudan University of Science and Technology. Informed consent was obtained from each participate after explained clearly the goal of study, any favorable outcomes and potential risk that might be encountered.

3.5.Methods

3.5.1.Collection of blood specimens

Five milliliter of blood was collected on EDTA container, after decontamination with ethyl alcohol, from the vein.

The plasma was separated from the cells by centrifugation at 3000 rpm for 5 minutes. The plasma was transported to Research Laboratory in ice bag then were stored at -70℃ until it was used.

3.5.2.Enzyme linked immunsorbent assay (ELISA)

3.5.2.1.Principle

The ELISA test kit provides a semiquantitative in vitro assay for human antibodies of the IgM class against hepatitis E antigens in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with recombinant antigens of hepatitis E virus. In the first reaction step, diluted patient samples are incubated in the well. In the case of positive samples, specific IgM anti bodies (also IgA and IgG) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgM (enzyme conjugate) catalyzing a color reaction.

3.5.2.2.Storage of ELISA kits

All kit contents were stored at 8℃ and used before expired data on the label.

3.5.2.3. Procedure

The following steps were done according to the instruction of the manufacturer (appendix 2)

3.5.2.3.1. Specimens preparation and dilution

The specimens were diluted 1:101 with buffer (provided with EUROIMMUN ELISA Kit) and mixed well by vortex.

3.5.2.3.2. Incubation

From each 3 calibrators, positive control, negative control and diluted plasma100 µl were added into the individual micro plate wells according to pipetting protocol. The plate was incubated for 30minutes at room temperature.

3.5.2.3.3. Washing

At the end of incubation period the plate cover was removed .The wells were washed manually with 300 µl of working strength wash buffer 3 times , wash buffer was left for 30 to 60 seconds per washing cycle, then the wells were emptied.

After the final washing cycle, the strip plate was turned onto clean towel, and tapped to remove any remainders.

3.5.2.3.4. Conjugate incubation

An amount of 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgM) was added into each of the microplate wells.

The plate was covered and incubated for 30 minutes at room temperature (18-25℃).

3.5.2.3.5. Washing

At the end of incubation period the plate cover was removed. The wells were washed manually with 300 µl of working strength wash buffer 3 times , wash buffer was left for 30 to 60 seconds per washing cycle, then the wells were emptied.

After the final washing cycle, the strip plate was turned onto clean towel, and tapped to remove any remainders.

3.5.2.3.6.Substrate incubation

From chromogen/substrate (TMB/H_2O_2) 100 µl was added into each of the micro plate wells and incubated for 15minutes at dark area.

3.5.2.3.7.Stopping of the reaction

Stop solution (0.5 M sulphuricacid) was added into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

3.5.2.3.8. Measurement

Photometric measurement of color intensity was made at a wavelength of 450 nm and reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution.

3.5.2.3.9. Calculation of the results

Results were evaluated semi quantitatively by calculating a ratio of the extinction value of the patient sample over the extinction value of calibrator according to following formula:

$$\frac{\text{Extinction ofthe cotrol of patient sample}}{\text{Extinction of calibrator}} = \text{Ratio}$$

3.5.2.3.10. Interpretation of the results

Ratio<0.8: Negative

Ratio≥ 0.8 to <1.1: Borderline

Ratio≥1.1: Positive

3.6. Data analysis

The data were analyzed using SPSS computer program(software version 16).

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

A total of 93 pregnant women who attended of Port Sudan Maternity Hospital in Red Sea State were enrolled in this study. The study was conducted during the period April to July to determine frequency of HEV.

The age range of pregnant women was between 15 and 45 years. The majority 48(51.6%) of study population were at age between 26-35 years (Table 1).

Regarding the gestational age, 19(20%) were at first trimester, 37(40%) were at second trimester and 37(40%) were at third trimester (Table 2).

The history of abortion was found in 12 (13%) participant (Table 3).

18 (19%) of the participant had a history of jaundice at least one in their life (Table 4).

Study on the detection of HEV among pregnant women was revealed that all blood samples were negative for IgM anti HEV antibodies, the frequency of HEV in pregnant women in Red Sea State is zero (Table 5).

Table 1. Frequency and percentage of pregnant women according to age

Age	Frequency	Percent
(15-25)	40	43%
(26-35)	48	51.6%
(36-45)	5	5.4%
Total	93	100%

Table 2. Frequency and percentage of pregnant women according to gestational age

Gestational age	Frequency	Percent
First trimester	19	20%
Second trimester	37	40%
Third trimester	37	40%
Total	93	100%

Table 3. Frequency and percentage of pregnant women according to history of abortion

Abortion	Frequency	Percent
Yas	12	13%
No	81	87%
Total	93	100%

Table 4. Frequency and percentage of pregnant women according to jaundice

Jaundice	Frequency	Percent
Yas	18	19%
No	75	81%
Total	93	100%

Table 5. Frequency and percentage of pregnant women according to laboratory findings

Lab finding	Frequency	Percent
Positive	0	0%
Negative	93	100%
Total	93	100%

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION

Hepatitis E virus (HEV) cause epidemic, especially in developing countries where hygiene is poor and many affected pregnant women suffer from hepatitis (Pal *et al.,* 2005).

IgM antibody to HEV in healthy subject has been used to measure the virus which is appears early during acute clinical illness but wanes over few months, so it detect acute HEV infection (Boccia *et al.,* 2006).

In the present study, the age range of pregnant women was between 15 and 45 years, the majority 48 (51.6%) of study population were at age between 26-35 years. Regarding the gestational age, 19 (20%) were at first trimester, 37 (40%) were at second trimester and 37(40%) were at third trimester, the history of abortion was found in 12 (13%), A history of jaundice was found in 18 (19%).

Investigation of plasma by ELISA technique revealed that all specimens free of HEV.

When compared with different previous studies, it founds to be lower than that reports from Africa which demonstrated a rate of 12% in Tunisia (Hannachi *et al.,* 2011)14% in Gabon (Caron and Kazanji.,2008) and Ghana (Adjei *et al.*,2009) for anti HEV IgM seroprevaleance among pregnant women.

In Sudan, fatality rate 17.8% was found during outbreak in Darfur, with rate of 31.1% among pregnant women (Baccia *et al.,* 2006).

Also less than in Khartoum State conducted by Alngashi, 2014 revealed lower rates of HEV infection (14.5%) and less than study conducted by Asma (9.9%) 2015.

Most of studied population were belonged to (26-35) age range 46(50.5%), most of studied pregnant women were in third trimester, and this result is in agreement with Resti *et al.*, 2014 study in Ahfaz State.

The differences of the relation between this study and risk factors. In other studies may due to variation in study sample size, study area, awareness of HEV infection among different population, different of food and water safety in different study area, and variation in sensitivity and specificity of the test performance.

5.2. Conclusion

The pregnant women in Red Sea State in this study are free from HEV: HEV is very rare among pregnant women in Red Sea State.

5.3. Recommendations

1. Further studies with large number of samples and more advanced technique are required to validate the results of the present study.
2. HEV screening must be checked with routine investigation for pregnant women, which help in early detecting and controlling any possible HEV complication for mother and baby.
3. Reinforce the importance of food safety and establish routine screening of HEV in food maker and all people work in food manufacture , this provide an important preventing agent to reduce the risk of HEV and other enteric infection for our community.

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Appendix

بسم الله الرحمن الرحيم

Appendix (1)

Questionnaire

Sudan University of Science and Technology

College of Graduate Studies

Title: Frequency of Recent HEV Infection among pregnant women in Red Sea State

Prepared by: Om kalthom Suliman Ahmed Ali

Supervisor Prof. Humodi Ahmed Saeed

Name……..Serial number…….

Age: 15-25( ) 26-35( ) 36-45( )

Gestational age:

First trimester ( ) Second trimester ( ) Third trimester ( )

History of abortion: Yas ( ) No ( )

Specimen: Plasma ( ) Other ( )

Method: ELISA IgM for HEV

Laboratory Findings:

Positive ( ) Negative ( )

Appendix (2)

EUROIMMUN Medizinische Labordiagnostika AG

Incubation

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrator, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash reagent wells 3 times with 450 µl working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgM) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to +25°C) (protect from direct sunlight).

Stopping the reaction:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

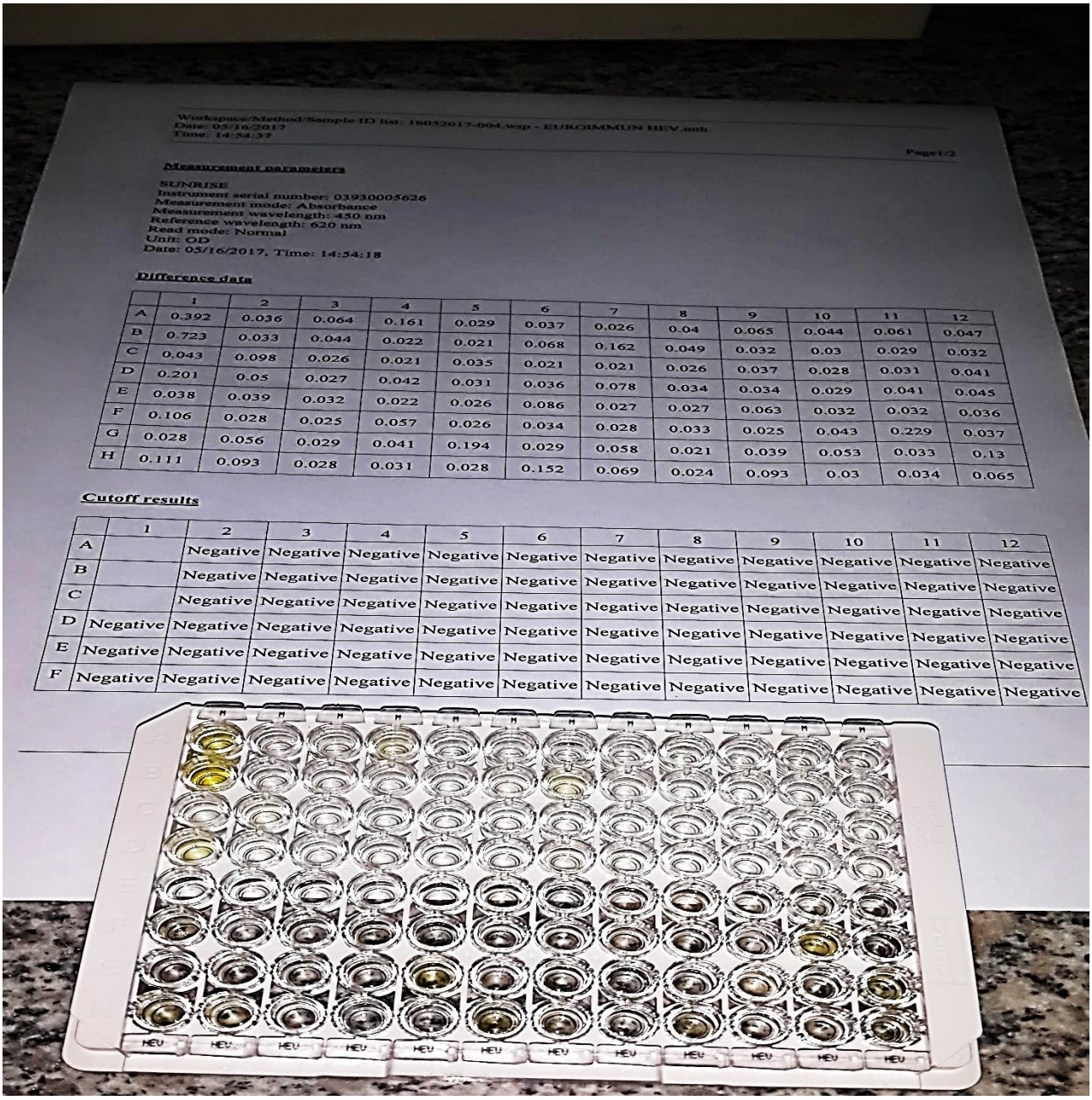
Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using the analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on inquiry.
Automated test performance using other fully automated, open system analysis devices is possible, however, the combination should be validated by the user.

Appendix (3)

HEV IgM Micro plate result



Workspace/Method/Sample ID list: 16052017-004.wsp - EUROIMMUN HEV.mth
Date: 05/16/2017
Time: 14:54:37

Page1/2

Measurement parameters

SUNRISE
Instrument serial number: 03930005626
Measurement mode: Absorbance
Measurement wavelength: 450 nm
Reference wavelength: 620 nm
Read mode: Normal
Unit: OD
Date: 05/16/2017, Time: 14:54:18

Difference data

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.392	0.036	0.064	0.161	0.029	0.037	0.026	0.04	0.065	0.044	0.061	0.047
B	0.723	0.033	0.044	0.022	0.021	0.068	0.162	0.049	0.032	0.03	0.029	0.032
C	0.043	0.098	0.026	0.021	0.035	0.021	0.021	0.026	0.037	0.028	0.031	0.041
D	0.201	0.05	0.027	0.042	0.031	0.036	0.078	0.034	0.034	0.029	0.041	0.045
E	0.038	0.039	0.032	0.022	0.026	0.086	0.027	0.027	0.063	0.032	0.032	0.036
F	0.106	0.028	0.025	0.057	0.026	0.034	0.028	0.033	0.025	0.043	0.229	0.037
G	0.028	0.056	0.029	0.041	0.194	0.029	0.058	0.021	0.039	0.053	0.033	0.13
H	0.111	0.093	0.028	0.031	0.028	0.152	0.069	0.024	0.093	0.03	0.034	0.065

Cutoff results

	1	2	3	4	5	6	7	8	9	10	11	12
A		Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
B		Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
C		Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
D	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
E	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
F	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative