Sudan University of Science and Technology

Collage of Graduate Studies

Seroprevelance of Hepatitis E virus(HEV) antibodies (IgG,IgM) among Sudanes Pregnant women in Dongola Maternity Hospital ,Northren Sudan

الكشف المصلي للأجسام المضادة لفيروس الكبد الوبائي (E)في الحوامل بمستشفى دنقلا للولادة,الولاية الشمالية

Adissertation submitted in partial fulfillment of the Degree of MSc in Medical Laboratory Science(Microbiology)

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Desember 2015

الآية

قال تعالى:

(الَّذِينَ يَذْكُرُونَ اللَّهَ قِيَامًا وَقُعُودًا وَعَلَى جُنُوبِهِمْ وَيَتَفَكَّرُونَ فِي خَلْقِ السَّمَاوَاتِ وَالْأَرْضِ رَبَّنَا مَا خَلَقْتَ هَذَا بَاطِلًا سُبْحَانَكَ فَقِنَا عَذَابَ النَّارِ)

صدق الله العظيم

سورة آل عمران الاية (191)

Dedication

To my lovely parents....

To my great brothrs(AHMED,MORTADA and

MOSAAB) and sisters..(SAFA and ESRAA)

The light of my life,my friends..

To all my Familly arround the world..

Acknowledgement

Firstly grateful thanks to ALMIGHTY ALLAH,for helping me in completing this research. I would like to acknowledge Prof.yousif Fadlalla Hamedelnil,for his unfailing patience,expert advices supervision, guidance and his valuable time.Extended thanks to college of Medical laboratory Sciences staff. My thanks and appreciation are extended to my colleagues and friends who strongly supported me throughout this study.A lot of thanks to the staff of Dongola Maternity Hospital .

Abstract

This study was descriptive and cross sectionalstudy, and aimed of this study was to determine the frequency of HEV antibodies (IgG,IgM) among pregnant women at Maternity Hospital of Dongola,Northren Sudan using Enzyme Linked Immuno sorbent Assay(ELISA).The study was conducted during the period of November 2015.Serum samples were collected and preserved at (-20C) until they performed.The study concluded that there was 13(14.44%)were positive for anti_HEV IgG ,while 77(85.56%)were negative,while all IgM samples showed seronegative IgM. 14(15.6%) with history of jaundies, 2(2.2%)
were anti_HEV IgG positive while 12(13.3%) were negative for anti_HEV IgG. 18(20%) were in first trimester ,3(3.3%) positive to IgG while 15(16.7%) negative to anti-HEV IgG,19(21.1%)were in second trimester2(2.2%), were positive to IgG while 17(18.9%)were negative to IgG,53(58.9%)were in third trimester,8(8.9%) positive to IgG,while 45(50%) negative to IgG,there was no significant correlation (*p* value> 0.05)between history of jaundice and trimester with seropositive IgG .8(8.9%)were illustrated ,1(1.1%)positive to IgG,while 7(7.8%)negative to IgG,36 (40%) were primarly school educated 3(3.3%)positive to IgG while 33(36.7%) negative to IgG,1(1.1%)was a middle school educated and she was negative to IgG ,25(27.8%)were high school educated,5(5.6)positive to IgG ,while 20(22.2%)negative to IgG,20(22.2%)were a university educated,4(4.4%)positive to IgG,while 16(17.8%)negative to IgG.36(40%)in rural area,2(2.2%)positive to IgG while 34(36.8%) negative to IgG ,54(60%) were in urban area 11(12.2%)positive to IgG ,while 43(47.8%) negative to IgG.31(34.4%)with history of miscarriage,5(5.6%)positive to IgG,while 26(28.9%)negative to IgG,59(65.6%)with no history of miscarriage

8(8.9%)positive to IgG,while 51(56.7%)negative to IgG, also there was no significant correlation (*p* value >0.05) between level of education , locality and history of miscarriage.The highest seropositive IgG found in age group between (26-35) and there was asignificant correlation (p value <0.05)between age groups and HEV-IgG seropositive .This study concluded that HEV antibody(IgG)has a low frequency among pregnant women,and recommended that the policy of screening for HEV antibodies in pregnant women in Sudan should be employed. Moreover, antenatal screening of pregnant women would ensure that treating clinicians could take further precautions to protect against perinatal HEV transmission (treated infected mothers,vaccine and educated the socity about the disease).also the tested for hepatitis E viruse shoud be performed in all acute hepatitis cases,especially those who negative to hepatitis B and C.

ملخص البحث

تعتبر هذه دراسة وصفية مقطعية تهدف لاجراء مسح لمعدل تواجد الاجسام المضادة.IgG,IgMوسط شريحة النساء الحوامل بمستشفى النساء والتوليد بدنقلا – الولاية الشمالية باستخدام جهازELISA،تمت الدراسة في فترة شهر نوفمبر 2015.جمعت العينات المصلية وحفظت في درجة حرارة(20C–)درجة مئوية الى أن تم فحصها. 13(14.44%)كانت نتائجهم ايجابية ل(HEV–IgG.) بينما كانت نتائج77(85.56%)سلبية ل(HEV–IgG.) لم تكن هناك اي نتائج ايجابية ل(HEV–IgM.) .(15.6%)14كان لديهم تاريخ مرضي باليرغان،2(2.2%)كانت نتائجهم ايجابيه ل IgG و12(13.3%)كانت نتائجهم سلبية لIgG.18(20%)كانو في الشهور الثلاث الاولى من الحمل،3(3.3%)كانت نتائجهم ايجابية ل IgG و15(16.7%)كانت نتائجهم سلبية لIgG،19(21.1%)كن في الشهور المتوسطة من الحمل،2(2.2%)كانت نتائجهم ايجابية لIgG.و17(18.9%)كانت نتائجهم سلبية لIgG. 53(58.9%)كانو في الشهور الاخيرة من الحمل،8(8.9%)كانت نتائجهم ايجابيه لIgG.و45(50%)كانت نتائجهم سلبية لIgG.(8.9%) لم يتلقوا التعليم،2(2.2%)كانت نتائجهم ايجابيه لIgG..بينما34(37.8%)كانت نتائجهم سلبية لIgG..36(40%)تلقوا تعليم اساسي،3(3.3%)كانت نتائجهم ايجابيه لIgG..بينما 33(36.7%)كانت نتائجهم سلبية لIgG..1(1.1%)تلقوا التعليم المتوسط وكانت النتيجه سلبية لIgG..25(27.8%)تلقوا تعليم ثانوي،5(5.6%)كانت نتائجهم ايجابية لIgG..بينما 20(22.2%) كانت نتائجهم سلبية لIgG.. 20(22.2%)تلقوا تعليم جامعي ،4(4.4%) كانت نتائجهم ايجابية لIgG..بينما 16(17.8%)كانت نتائجهم سلبية لIgG.36(37%) يسكن في المناطق الريفية خارج دنقلا،2(2.2%)كانت نتائجهم ايجابية لIgG..بينما 34(37.8%) كانت نتائجهم سلبية لIgG.54(60%)يسكنون في داخل مدينة

دنقـــلا،11(12.2%) كانـــت نتـــائجهم ايجابيـــة لIgG.بينمـــا 34(37.8%)كانـــت نتـــائجهم ســـلبية لIgG. 31(34.4%)لـــديهم حالـــة اجهـــاض ســـابق،5(5.6%)كانـــت نتـــائجهم ايجابيـــة لIgG.بينمـــا 26(28.9%)كانـــت نتـــائجهم ســـلبية لIgG..59(65.6%) لـــيس لديهم حالة اجهاض سابق .

لاتوجـــد فروقـــات ذات دلالـــة احصـــائية بـــين وجـــود الاجســـام المضـــادة مـــن النـــوع.IgG وIgM ومســـتوى التعلـــيم ،ووجـــود تـــاريخ مرضـــي باليرغـــان،ووجود حالـــة اجهـــاض سابقة ،ومكـــان الاقامـــة فـــي الريـــف أو الحضـــر وعمـــر الحمـــل ،(القيمـــة الاحتماليـــة أكبـــر مـــن 0.05).أعلـــى معـــدل لتواجـــد الاجســـام المضـــاده وجـــد فـــي الفئـــة العمريـــة مـــابين (26-35)بنســـبة 11.11%،وهنـــاك فـــرق ذو دلالـــة احصـــائية (القيمـــة الاحتماليـــة أقـــل من 0.05)بين النتيجة الايجابيةل HEV-IgG وبين هذه الفئة العمرية.

خلصـــت هـــذه الدراســـة الـــى أن هنـــاك انخفاضـــا نســـبيا فـــي وجـــود الاجســـام المضـــادة لالتهاب الكبـــد الوبـــائي مـــن النـــوع (E) فـــي دم النســـاء الحوامـــل ،وأوصـــت هـــذه الدراســـه باعتماد الكشـــف عـــن الاجســـام المضـــاده لالتهـــاب الكبـــد الوبـــائي كفحـــص روتينـــي للنســـاء الحوامل أثنـــاء متابعـــة الحمـــل مـــع معالجـــة الاصـــابات واعطـــاء التحصـــين والتثقيـــف العـــام لهن.أيضـــا اعتمـــاد الكشـــف عـــن الاجســـام المضـــادة لالتهـــاب الكبـــد الوبـــائي مـــن النـــوع (E) للذين لم تثبت اصابتهم بالتهاب الكبد الوبائي من النوع (B) و(C) .

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List of Abbreviations

aa	amino acid
aHEV	avian Hepatitis E Virus
AJS	Acute Jaundice Syndrome
ALF	Acut Liver Failuer
ALT	Alanine Transferase
AST	Amino Transferase
AVH	Acut Viral Hepatitis
cDNA	cloned Deoxyribo Nucelic Acid
CFR	Case Fatality Rate
DIC	Disseminated Intravascular Coagulation
DILI	Drug-Induced Liver Injury
DNA	DeoxyriboNucleic Acid
ELISA	Enzyme Linked Immuno Sorbant Assay
FMIA	Fluorescent Microbead Immuno Assay
HEV	Hepatits E Viruse
HEV-LP	Hepatitis E Virus Llike Particles
HRP	Hourse Redish peroxidase
IgG	Immuneo globulin G
IgM	Immunoglobulin M
kb	kilo bases
mHEV	mammalian hepatitis E virus
mRNA	messenger ribo nucleicacid

NCR	Non Coding Region
nm	nano meters
NND	NeoNatal Deaths
ORF	Open Reading Rrame
PCR	Polymerase Chain Reaction
PK	Proteinase K
RdRp	RNA dependent RNA polymerase.
RNA	RiboNucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction

Chapter One
Introduction

Introduction

Hepatitis E virus, a single-stranded RNA virus, was first described in 1983 as spherical, 27-30nm virus-like particles.The HEVgenome is a7.5-kb,composed of three open reading frames(ORF), ORF1,2,and3,which encode the non structural proteins,the Capsid protein,and acytoskeleton-associated phosphor protein, respectively. Analysis of its RNA helicase and RNA-dependent RNApolymerase regions shows that HEV forms a phylogenetically distinct group that was recently placed into a separate genus: Hepevirus.Hepatitis E virus (HEV) is the major etiologic agent of enterically transmitted non-A, non-B hepatitis in humans worldwide, and an emerging infectious agent where it can cause acute viral hepatitis worldwide , with estimated 20 million cases of HEV infection occur globally and 70, 000 deaths.(Eltayeb *et al* .,2015).

Hepatitis E virus(HEV),identified over 30 years ago,remains a serious threat to life ,health, and productivity in developing countries of Asia, Middle East, and Africa ,as well as in Mexico , these continue to occur in locations with poor sanitary infrastructure ,and are common place in refugee camps,such as the January 2013 out break in southern Sudan .

Geno type1(HEV-1)is the Primary cause of epidemic and sporadic cases of hepatitis E in Developing countries in Africa and Asia,where it is transmitted primarily through fecally contaminated water supplies.(Krain *et al*.,2014),in industrialized countries including Japan,United States, and countries in Europe hepatitis E is considered as an emerging disease of global importance , and has been diagnosed only among travelers returning from HEV-endemic countries.Most autochthonous cases occurring in industrialized countries are due to the genotype 3, where as genotypes 3 and 4 are zoonotic. Pigs are aknown reservoir for HEV,and several other animal species,including deer

Rabbits and mongooses,may potentially serve as HEV reservoir. (Cao and Jin.,2012) .Due to hormonal and immunological changes, pregnant women are more prone to have severe form of HEV infections where there is increasing evidence that HEV is an important contributor to maternal and perinatal morbidity and mortality, especially in the developing countries (Eltayeb *et al*.,2015).Patients with acute hepatitis E present with typical acute hepatitis symptoms such as dark urine. Typical biochemical changes in acute HEV patients include increased serum levels of alanine/aspartate aminotransferases (ALT and AST) with abnormal liver function.

Serological diagnosis of acute infection is based on enzyme immunoassy (EIA) tests. IgMHEVis usually detectable at the onset of symptoms(and maximum thre e months after).IgG reaches a peak shortly afterinfection and can persist for years Also Laboratory diagnosis of the pathogen relies on direct detection of one of its components (i.e., nucleic acids or proteins). Detection of the HEV capsid protein has been attempted using a sandwich enzyme immunoassay.Molecular technique is the major critical issues for diagnosis HEV including RT-PCR.

1.1 Rationale

In Sudan, a high mortality rate was reported among pregnant women in an outbreak of HEV in Darfur and in eastern Sudan(Eltayeb *et al*., 2015),also there is arecent study from Medani in central Sudan, located in Al Gezira state, in central Sudan.There is no reports of HEV seroprevelance among pregnant women from Northern Sudan,so this study provid a policy makers by primarly information about HEV infection among pregnant women to ensur that the screening of HEV will become apart of antenatal care programme and develoing aprevention measures.

1.2Objectives

1.2.1 General objective

To determine seroprevelance of HEV-IgG/IgM antibodies in pregnant women in Northren State.

1.4 Specific objectives

1.To detect the two types of HEV antibodies(IgG,IgM) among pregnant women in Northren Sudan by using ELISA technique.

2.To determine the relationship between seropositive IgG,IgM of HEV and possible risk factors such as (history of jaundies,history of miscarriage,level of education,residence,age,gestational age).

Chapter Tow

Literature Review

Literature Review

2.1 Hepatitis E virus

Hepatitis E virus, a single-stranded RNA virus, was first described in 1983 as spherical, 27-30 nm virus-like particles. Hepatitis E virus (HEV) infection results in hepatitis E, an acute and self-limited disease. The virus is transmitted in a faecal–oral manner and is a major cause of viral hepatitis in much of the developing world, In industrialized countries, hepatitis E is considered as an emerging disease of global importance , and diagnosed only among travelers . Most autochthonous cases occurring in industrialized countries are due to the genotype 3.

A curious feature of hepatitis E is the unusually high rates of mortality that are observed in pregnant women, in whom the disease is exacerbated by the development of fulminant liver disease .The disease was first recognized in the Indian subcontinent in the 1950s.Initially thought of as hepatitis A infection, it took almost 30 years to recognize it as different virus when the sera from persons during two water-born epidemics in India were negative for hepatitis A and B (Navaneethan *et al* .,2008).

2.2 Historical background

HEV-1 out breaks affecting tens of thousands of people in Central, South,and East Asia have been documented since the1950s,the Largest known HEV epidemic to date,occurring from 1986 to1988 in the Xinjiang region of China sickened over119,000 people and resulted in 707 documented fatalities,414 of whom were pregnant Women.Genotype-2(HEV-2) was first identified from cases in out- breaks in two rural towns in Morelos ,Mexico in 1986 and 1987 other HEV-2 strains have appeared in Africa, where they have also been implicated in outbreaks (Krain *et al*.,2014).Biological time-clock studies suggest that HEV

diverged in to four genotypes about 500 years ago.Although HEV was only discovered recently,it has probably caused disease in humans for centuries.Historical descriptions of outbreaks of jaundice and deaths in pregnant women suggest that in the 19th and early 20th centuries,hepatitis E was presentin Germany ,France , Italy and the Balkans (Daltona *et al*., 2013).

2.3 Classification of HEV

HEV was originally classified in the family Caliciviridae. However, because the HEV genome does not share significant sequence homology with caliciviruses the virus was subsequently declassified from the family Caliciviridae ,but on the basis of comparative phylogenetic analysis ,it was recently removed from the caliciviridae family and assigned as an un classified genus of hepatitis E-like virus.(Wan *et al* ,2001).Currently,HEV is placed in asole genus Hepevirus within anew family Hepeviridae, the genus Hepevirus includes the four recognized major genotypes ofHEV in mammalian species: genotype1(Burmese-like Asian strains) , has been further classified into four subtypes and most of them have been grouped into genotype 1A. A subgenotype shift, may have been responsible for the different geographical morbidity in pregnant women in Southern India and Egypt. (Jin.,2010).Genotype 2 (a single Mexican strain and some African strains), genotype 3 (strains from sporadic human cases in industrialized countries, and animal strains from pigs, deer, and mongeese), and genotype 4 (strains from sporadic human cases in Asia, and swine strains from pigs (Jin.,2010).Despite the variety of genotypes, only two serotypes of HEV have been reported to date. Serologic tests cannot distinguish between infection with different HEV genotypes (Emerson et al., 2005).Broad environmental,

dietary,and overall health differences across settings and limited information on the distribution of viral subgenotypes in many parts of the world make it difficult to know whether HEV-1 and HEV-2 are inherently more virulent in Humans than HEV-3 and HEV-4 or whether the occurrence of epidemics and the incidence of illness and death reflect primarily exposure-and host-related risk factors. (Krain *et al*.,2014).Two major species of HEV have been recognized,Mammalian HEV(m HEV) causes acute hepatitis in humans and has animal reservoirs in pigs and possibly other mammals, where as avian HEV (a HEV) ,is associated with hepatitis-splenomegaly syndrome in chickens.Avian HEV is currently classified as aseparate,floating species in the Hepeviridae family, because of the extensive sequence variation(approximately 50%)between avian and mammalian HEVs,at least three genotypes of avian HEV have been identified from chickens worldwide .(Cao and Meng.,2012) .

2.4 Morphology:

The crystal structure of HEV-like particles (HEV-LP)consisting of capsid protein was determined at 3.5-Å resolution.The capsid protein exhibited a quite different folding at the protruding and middle domains from the members of the families of Caliciviridae and Tombus viridae,while the shell domain shared the common folding. The structural and biological findings are important for understanding the molecular mechanisms of assembly and entry of HEV and also provide clues in the development of preventive and prophylactic measures for hepatitis E.(Yamashit *et al*.,2009).Electron microscopy of human stool specimens showed that HEVis anon enveloped sphericall particle with adiameter of Approximately 320Å . The truncated HEV capsid protein has 3 definite domains designated as S (shell), M (middle),and P(protruding), composed of the amino acid residues 129–319 ,320–455,and 456–606,respectively.

2.5 HEV genome

Hepatitis E virus genotypes are important as they correlate with the severity of infection.The viral genome consists of a short 5' non coding region (NCR) and a 3'NCR, and three partially over lapping open reading frame(ORFs) ,encodes at least three proteins .Open reading frame1(ORF1),structural proteins.The protein encoded by open reading frame 2 (ORF2) is the major HEV capsid protein involved in the assembly of theHEV particle in cytoplasm.The protein encoded by open reading frame 3 (ORF3) encodes a small phosphorated protein that is associated with cytoskeleton and appears to be involved in virushost interactions. (Cao and Meng.,2012).

2.6 Viral Replication:

Step a:HEVattaches to the cell surface via HSPGs,HSC70 or other putative attachment receptor(s)and then enters the cell via aunknown specific cellular receptor. Step b:The HEV virion penetrates the membrane and enters the cells HSP90 and Grp78may be involved in this transport .The virion then uncoats and releases the positive-sense genomic RNA in to the cytoplasm of the cell . Step c Thepositive-sense genomic viral RNA serves as the template to translate the ORF1 nonstructural polyprotein in the cytoplasm. Step d:The viral RdRp synthesizes an intermediate,replicative negative-sense RNA from the positive sense genomic RNA that (stepe) serves as the template for the production of positive-sense ,progeny viral genomes. Step f:The ORF2 and ORF3 proteins are translated from the subgenomic,positive stranded RNA, and step g: the ORF2 capsid protein packages the genomic viral RNA and assembles new virions. Steph:The nascent virions are transported to the cell membrane.The ORF3 protein facilitates the trafficking of the virion,and(stepi) then ascent virions are released from the infected cells. (Cao and Meng.,2012).

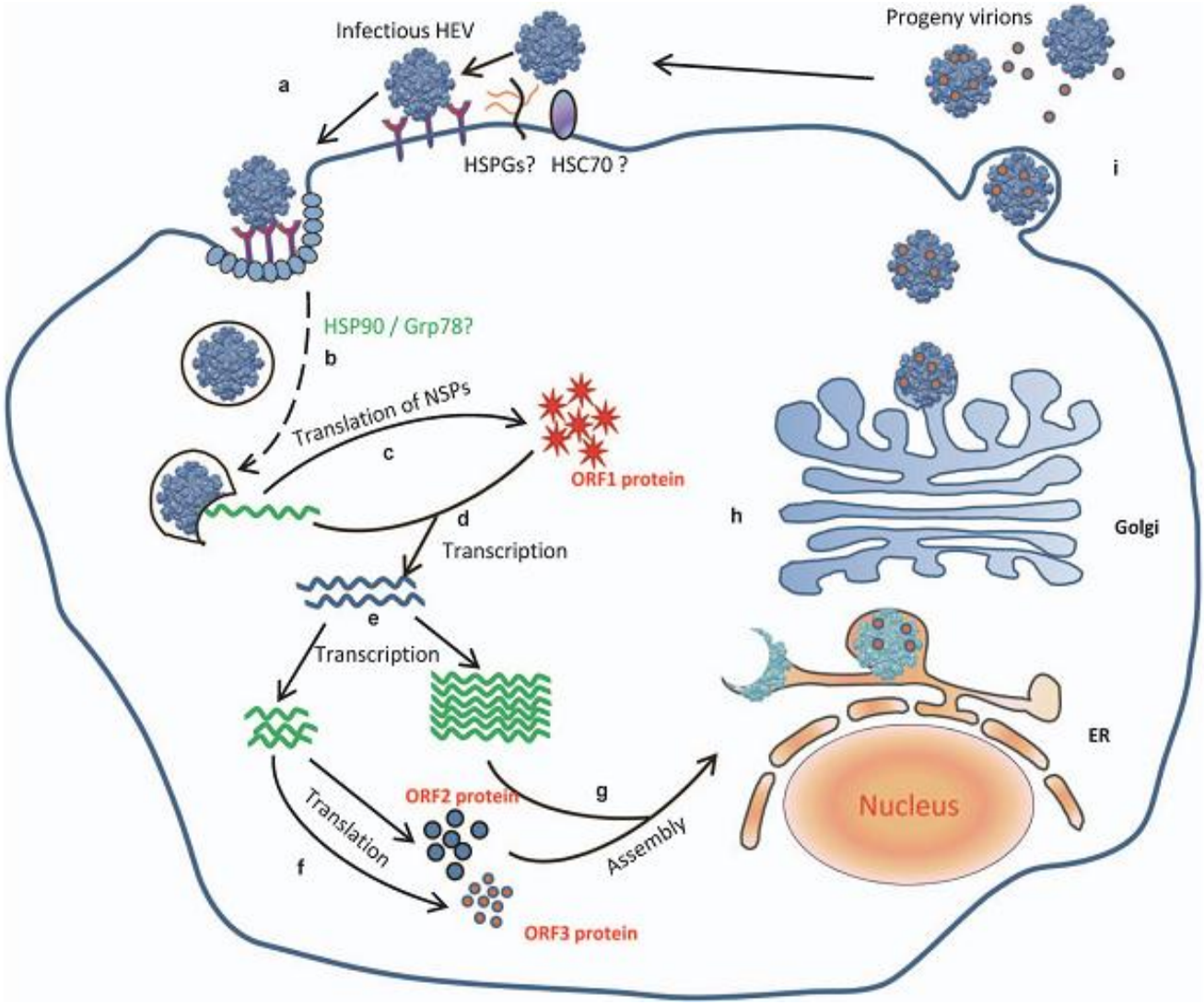

Figure1: Proposed life cycle of HEV,modified from Ref.(Jameel., 1999)

2.7 Transmission

HEV infection transmitted through drinking of fecally contaminated water. Zoonotic transmission of HEVgenotypes 3 and 4 to humans can occur by consumption of contaminated meat or meat products or by contact with infected animals.The virus has been found in both domestic and wild animals.Humans are the natural host forHEV,but a nonhuman reservoir for HEV is also possible.Non-human primates (e.g. chimpanzees, cynomolgus monkeys, rhesus monkeys, pig-

tail monkeys, owl monkeys, tamarins, African green monkeys),are susceptible to natural infection with humans HEV strains.Person-to-person transmission of HEV appears to be uncommon; however, nosocomial transmission, presumably by person-to-person contact, has been reported to occur.Hepatitis E cases in most of the world peak in early to mid-adulthood , with both antibody prevalence and attack rates of clinical illness highest among adults and little overt disease in children Intra familial transmission of HEV appears relatively uncommon,though some evidence of household transmission has been reported.Slums and refugee camps are particularly vulnerable to outbreaks as access to clean water and sanitary waste disposal facilities is often limited.(Krain *et al*.,2014).The possibility of blood borne transmission of hepatitis E especially in endemic areas are found .Vertical transmission of HEV infection from mother to infant is known,it is not considered to be an important route of transmission for HEV. However, some evidence shows that such transmission of HEV occurs and carries appreciable perinatal morbidity and mortality.(wan *et al*.,2001).

2.8 Immune response and Immunity:

The patterns of IgG-class and IgM-class antibody responses during HEV infection. These responses are typical of those detected with the 55 to 63 kd ORF2 antigens.Typically, both IgG and IgM antibodies are detectable at the onset of disease, IgM declines to undetectable levels over a period of 2 to 6 months, and an approximately 10-fold decline in IgG levels is seen over this period.levels then stabilize.IgA responses to HEV (as a correlate of mucosal immunity) have been detected in around 50% of patients these antibodies rapidly declined to undetectable levels, although IgA may persist somewhat longer than IgM.The role of IgA in immunity to HEV infection is unknown, but because passive immunization with IgG appears to be suffcient for protection,IgA is

likely not essential.Until recently,there were few laboratory-confirmed reports of reinfection with HEV among immune competent persons,which suggested the possibility of broad,enduring protection following resolution of natural infection serologic evidence and animal studies suggest that infection with one strain of HEV confers cross-protection to other strains, even a cross genotypes 1 to 4. (Krain *et al*.,2014).

2.9Clinical features

The incubation period ranges between 14 and 60 days (mean 30to40 days). The ratio of symptomatic to asymptomatic infection has been reported to range from 1:2 to 1:13. In most patients,hepatitis E causes a self limiting illness which lasts afew weeks. Following an incubation period symptoms of hepatitis develop with fever and nausea followed by abdominal pain,vomiting, anorexia,malaise and hepatomegaly.Jaundice occurs in about 40% of patients (Kamar.,2014).

Chronic hepatitis E infection is rare and has only been reported in organ transplant patients and HIV patients,but within the past few years, HEV has been responsible for chronic hepatitis, which can rapidly evolve to cirrhosis, in immunocompromised patients .Fulminant disease is rare also except in pregnant women. However, for pregnant women, hepatitis E is more serious, maternal fatality of 16%to20% had been reported when hepatitis E occurs in the third trimester of pregnancy and is caused by acute liver failure,eclampsia,or hemorrhage (Kwon and Anna.,2014).The association between neurologic signs and symptoms neurologic(e.g., Guillain-Barré syndrome, neuralgic amyotrophy acute transverse myelitis) and HEV infection has been based on detection of anti-HEV immunoglobulin (IgM) in serum.(Kamar *et al*.,2011)

2.9.1 Acute hepatitis E:

After an incubation period most cases of acute hepatitis E present with symptoms indistinguishable from other forms of viral hepatitis .In most cases,the patients recover within4–6weeks,but patients with underlying chronic liver disease have apoor prognosis with excess mortality from sub acute liver failure infection is usually asymptomatic with modestly elevated liver function tests,and has only been observed with HEV geno type 3(Dalton *et al*.,2013)

2.9.2 Extra-hepatic manifestations of hepatitise:

Hepatitis E has been shown to produce arrange of extra-hepatic manifestations including acute pancreatitis ,thrombocytopenia,aplasticanaemia,acute thyroiditis and glomerulonephritis.(Dalton *et al*.,2013)

2.10 Clinical finding

Liver function tests are an important adjunct to diagnosis of acute HEV. The serum aminotransferases ALT and aspartate amino transferase(AST) show variable increases during the prodromal phase. The ALT level peaks at the onset of symptoms before the serum bilirubin begins to increase. Peak levels of ALT vary from 1000 U/L to 2000 U/L at the onset. ALT levels progressively diminish during the recovery phase. Some patients with anicteric acute HEV infection have only raised ALT levels.Jaundice is visible in sclera or skin when the serum total bilirubin level exceeds 2.5 mg/dL, usually following the peak levels of ALT. Peak serum total bilirubin levels range from 5 to 25 mg/dL; both conjugated and unconjugated fractions are increased. In cholestatic HEV infection (around 10% of patients), serum bilirubin may remain elevated for prolonged periods.Prothrombin time may be increased in acute viral hepatitis

especially in fulminant hepatitis, indicating extensive hepatocellular necrosis and a worse prognosis. Similarly, a reduction in the serum albumin level may occur. Reduced blood glucose levels leading to hypoglycemia may be observed in patients with prolonged nausea, vomiting, and inadequate carbohydrate intake. Neutropenia, lymphopenia and atypical lymphocytes may occasionally be observed during the acute phase of viral hepatitis**.**

2.11 Epidemiology of hepatitis E virus

HEV has at least 2 distinct epidemiological profiles: (1) large outbreaks and epidemics in developing countries, usually caused by HEV genotype 1, resulting in high morbidity and mortality among pregnant women and young children and (2) very few symptomatic cases of HEV genotype 3, most cases without symptoms or clear source(s) of infection, but frequent seroreactivity in 5%–21% of asymptomatic persons in developed countries (Teshale *et al.*,2010).Human illnesses were epidemiologically linked to consumption of undercooked pork liver and consumption of raw deer meat and raw wild boar liver was responsible for outbreaks of hepatitis E.Until 1997, hepatitis E was thought to occur only in developing countries including Africa, central Asian republics of the former Soviet Union, Afghanistan , Bangladesh , Borneo, Burma ,China India , Mexico Mongolia , Nepal , Pakistan , Thailand ,Vietnam and some parts of the Middle East.(CDC 1993, Meng 1999, 2000). Hepatitis E was not thought to be endemic in developed countries, including the U.S. Since 1997, HEV has been documented in humans and swine in many countries previously considered nonendemic, including Argentina, Australia Austria, Canada, Germany,Greece Japan, Korea, the Netherlands, New Zealand, Spain, and Taiwan .Taking aparticularly high toll in pregnant women and their fetuses, HEV has infected human populations In 28 of 56 African countries. Since1979 ,17 HEV out

breaks have been reported about once every other year from Africa causing a reported 35,300cases with 650 deaths(Hoon *et al*.,2014) .The prevalence of HEV infection is between 7.2% and 24.5% in developing countries with poor hygiene conditions, while the rate ranges around 0–3% in developed countries(Oncu *et al*.,2006).The largest documented Out break took place in north west Chinain1986–1988 involving approximately 120,000 cases.Arecent study has estimated that in nine of 21 Global Burden of Disease regions,there are 3.4 million symptomatic cases of hepatitis E each year,with 70,000 deaths and 3000 still births (Daltona., 2013).

2.12 HEV in Sudan

There are few published data on the seroepidemiology of HEV infection in pregnant women and none are available from Sudan specifically,a high mortality rate was reported among pregnant women in an outbreak of HEV in Darfur and in eastern Sudan (Boccia *et al*., 2006; Rayis *et al*., 2013).

2.12.1 In Khartoum state:

HEV IgG antibodies were detected in 41.1% (37/90). The highest percentages were recorded in the second and third trimesters of pregnancies (37.8% and 48.7%) respectively. (Ahmad *et al*,2014)

2.12.2 In Darfur State:

Acase:fatality ratio of 17.8% was found in an outbreak in Darfur, with a ratio of 31.1% among pregnant wome(Adjei *et al*., 2009).Mornay camp Almost one-quarter of the patients with hepatitis E admitted To the hospital were pregnant women,and among these ,one third died , the case-fatality ratio among pregnant women admitted to the hospital was almost twice that of other hospitalized

patients with HEV infection(42%vs.18%).This high case-fatality ratio among pregnant women,although still unexplained(Boccia *et al*.,2006).

2.12.3 In Al Gezira State:

The high prevalence (12.1%) of HEV IgG antibodies among pregnant women regardless of their age, parity, residence and educational levels. Two hundred and nine women were enrolled in that study. There was no signifcant difference in the age, parity, education, gestational age, BMI or history of miscarriage between seropositive and seronegative anti-HEV IgG women (Eltayeb *et al.,* 2015).

2.12.4 Kassala State:

During the 4 months of the HEVepidemic, 39 pregnant women presented at the hospital with various symptoms.There were11(28.2%)maternaldeaths,14(36.0%) intra uterine fetal deaths,and eight (20.5%) cases of post partum haemorrhage There were nine (23.0%)cases of preterm (<37weeksofgestation) deliveries. Fulminant hepatitis with hepatic encephalopathy was the most common cause of death among these patients(Rayis *et al*,2013).

2.12.5 Southern sudan:

A cross-sectional serosurvey conducted in Jamam Camp(Jamam refugee camp Maban County, Upper Nile State, South Sudan), in november 2012 indicated that 54.3% of the population was susceptible to HEV infection. Across all camps an(AJS) Acute jaundice syndrome case-fatality rate (CFR)of10.4% was observed among pregnant women.(Thomas *et al*.,2013)

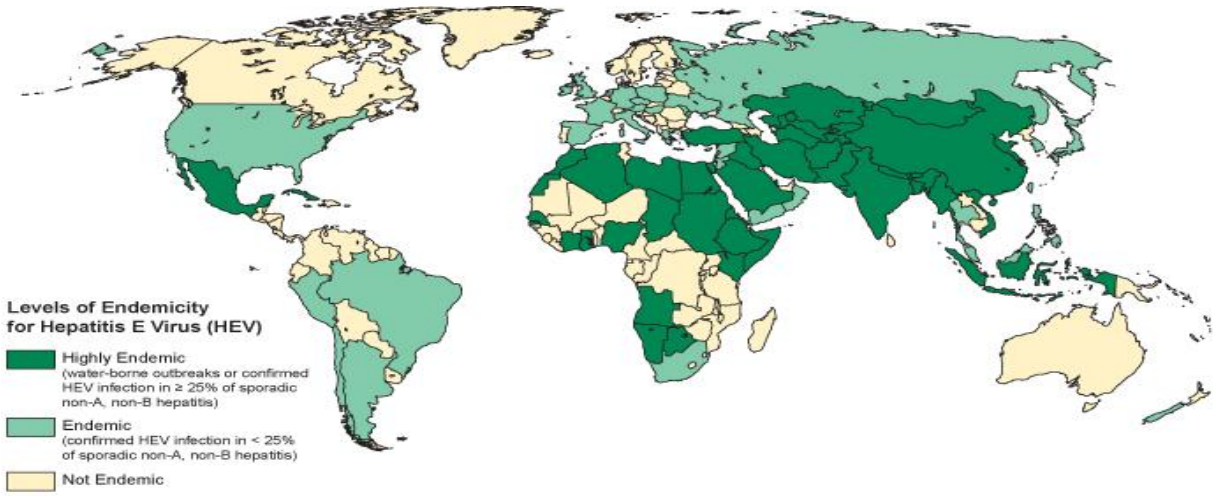

Figure2: World wide distribution of hepatitis E virus in 2008 .Courtesy of Dr.Chong-GeeTeo,and created for use by the Centers for Disease Control And prevention CDC,2012.(Teshale *et al*.,2010).

2.13 HEV and pregnant women

Although vertical HEV infection is common and can lead to a high neonatal mortality, HEV is a self-limiting infection in survivors with short-lasting viremia (Khuroo.,1991 and Krawczynski.,1993).In pregnant women, Hepatitis E has both a high incidence and a severe course in some geographical regions of HEV-endemic countries, such as Northern India,often leading to fulminant hepatic failure and death ,while in other HEV-endemic countries, such as Egypt, it has been shown to have a benign course with little or no morbidity . The high mortality rate in pregnancy has been thought to be secondary to the associated hormonal changes during pregnancy (Levels of progesterone,estrogen and human chorionic gonadotropin (HCG) increase with pregnancy that steroid hormones may influence viral replication) and consequent immunological changes, the prevalence and severity of HEV infection did not differ significantly in various stages of gestation (Navaneethan *et al* .,2008)

During pregnancy, the maternal immune system is clearly altered to tolerate a genetically different fetus. T-cells are markedly reduced during early pregnancy up to the 20th week of gestation, leading to a reduced level of immunity. (Krain *et al*.,2014). In addition to the above-mentioned factors, suggested that infection of the fetus with HEV may be responsible for the increased severity of the disease in the mother (kumar and Begum., 2010).

Pregnant women die of obstetric problem including hemorrhage or eclampsia , or develop fulminant hepatic failure. Stillbirths are common,as is vertical transmission to infants ,excess mortality in pregnancy with HEV genotypes 1and2 is unique,it is not seen with genotypes 3 and 4. (Kamar,2011).

2.14 Fetomaternal outcome

Pregnant women, particularly those in the second and third trimesters, are more frequently affected during hepatitis E outbreaks, and have worse outcomes mortality rates ,especially those infected in the third trimester, range from 15% to 25% (Oncu *et al*.,2006).Among pregnant women, these illnesses can lead to coagulation defects,postpartum haemorrhage, organ failure and high maternal mortality and poor outcomes of their newborns such as still births, neonatal deaths (NND), acute and chronic liver disease and hepatocellular carcinoma. The various maternal complications associated with viral hepatitis are preterm labour obstetric haemorrhage, fulminant hepatitis, hepatic encephalopathy, renal failure DIC and death. The various neonatal complications are intrauterine death prematurity and risk of vertically transmitting the hepatitis infection (Shukla *et al*.,2011).

2.15 Diagnosis

2.15.1 Serological diagnosis:

Is based on enzyme ,Enzyme ImmunoAssay(EIA).

2.15.1.1 Ab detection

(ELISA):Enzyme Linked Immunosorbant Asssay:

For the determination of IgM antibodies to Hepatitis E Virus in human plasma and sera.Microplates are coated with HEV-specific synthetic antigens encoding for conservative and immunodominant determinants derived from Mexican and Burma virus strains.The solid phase is first treated with the diluted sample and anti HEV IgM are captured, if present, by the antigens adsorbed on wells. anti HEV IgM antibodies are detected by the addition of polyclonal specific anti hIgM antibodies, labeled with peroxidase (HRP).The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HEV antibodies present in the sample.

The ELISA-based methodologies are not able to offer any epidemiological data about the distribution of the different variants of the virus. The determination of subtypes is usually performed by analyzing the nucleic acid sequence, namely by sequencing either the whole genome or a region that is informative for subtyping (Zhai *et al*., 2006).

2.15.1.2 Ag detection:

Antigen detection for both pathogen detection and indication of the acute phase of hepatitis E and screening for HEV-positive blood donors,both in the acute

phase and during the period after seroconversion of anti-HEV.(Antigen detection protocol for the sandwich ELISA+RNAdetection)

Thus, in endemic regions, it is better to use only ORF-3 based ELISA tests.The most sensitive and specific test to diagnose acute HEV infection is the detection of HEV RNA in the sera by RT-PCR.(Kamar,2014).

2.15.2Molecular techniques:

2.15.2.1 PCR:

Polymerase chain reaction (PCR) detect HEV RNA in serum and stool, (Viremic phase of 4-6 weeks with nucleic acid being Detected up to 112days.).Longer duration of nucleic acid detection has been reported in immunocompromised organ-transplant recipients following acute HEV.PCR is a widely used technique in molecular biology because of its high sensitivity specificity ,It is a cycling reaction where each cycle contains denaturation, annealing and extension that results in an exponential amplification, producing vast amount of DNA at the end of the procedure,to identify the PCR product gel electrophoresis with ethidium staining is used for size separation.

(Gyarmati.,2008).

Because of the high variability of HEV genome, a universal detection was proved to be difficult for years,to target the virus with ligation-based probes needed a stable identification system in advance,for this purpose, two real-time assays were developed, both targeting the same ORF2-3 regions. The TaqMan assay was chosen because of its reliability, prevailing and widespread nature; the PriProET assay was chosen because of its reported higher tolerance for mismatches(Gyarmati *et al*.,2007).

2.15.2.2 Detection of RNA:

The amplification of HEV RNA by reverse transcription (RT) followed by the PCR of the cDNA is the most sensitive technique to screen clinical specimens during the course of the disease,this method can identify active infection and help confirm serologic results. HEV RNA was extracted from the fecal suspensions and from serum samples by amodified proteinase K(PK) method. Sense and anti sense synthetic oligonucleotide primers corresponding to the nucleotide sequence of the putative HEVRNA polymerase gene were used for RT-PCR. Finally,the PCR products were analyzed by electrophoresis in a 2% agarose gel followed by ethidium bromide staining ,and the specificity of the band was checked by Southern blotting and hybridization witha 32 P-random priming labeled HEV plasmid(Turkoglu *et a.,l* 1996)

2.15.3 Cell culture:

The establishment of a practical cell culture system that facilitates the propagation of HEV invitro is critical for virological characterization,as wellas for studies on the prevention of HEV infection. The lack of an efficient cell culture system for Hepatitis E virus(HEV)has greatly hampered detailed analyses of this virus.The first efficient cell culture systems for HEV that were developed were capable of secreting infectious HEV progenies in high titers in to culture media,using PLC/PRF/5cells derived from human hepatocellular carcinoma and A549 cells derived from human lung cancer as host cells.(Okamoto.,2013).

2.15.4 Other laboratory tests:

Serum alaninetransaminase,albumin,bilirubin(total+direct),alkaline phosphatase and g_glutamyl trans peptidase were measured with asequential multi auto analyzer(Chunlin.,*et al* 2000)

immunofluorescent antibody blocking assays to detect antibody to HEV antigen in serum and liver and immune, electron microscopy to visualize viral particles in feces also other methods for diagnosis.

2.16 Treatment

No therapeutic measures have been proven effective following the onset of disease. Patients are typically advised to rest, get adequate nutrition and fluids and avoid alcohol. Hospitalization is sometimes required in severe cases and should be considered for pregnant women.Patients with severe Hepatitis and under lying chronic liver disease have a poor prognosis.A number of such patients have been treated successfully with ribavirin.Immuno suppressed patients with chronic infection should be treated,as with out treatment they may develop rapidly progressive cirrhosis. In transplant recipients,the first step is to reduce the dose of immune suppressive therapy,if possible .This will achieve viral clearance in up to30%.If this is ineffective,or is not possible because of organ rejection,then a 3-month course of ribavirin will clear HEV in the majority of patients .(Daltona ,2013).

2.17 Prevention and Control :

Prevention of hepatitis E relies primarily upon the provision of clean water supplies. Educational programs should be designed to stress sanitary disposal of feaces and careful hand washing after defecation and before handling food. Travelers to endemic areas should take precautions to avoid contaminated food and water, including avoidance of drinking water, eating uncooked shellfish and uncooked fruits or vegetables not peeled or prepared by the traveler.HEV may be wide spread among pregnant women in Sudan and it may circulate in the general population.Therefore,apopulation-based study to confirm or exclude this

speculation is urgently required.Inaddition,the sanitary conditions under which pregnant women live and work need to be improved.(Rayis *et al*,2013)

2.18Vaccine

There is currently no FDA-approved vaccine for hepatitis E but two recombinant HEV vaccine candidates have been clinically evaluated, one vaccine candidate,rHEV,showed 95.5% efficacy after three doses in phase II trial in Nepalese military population while the other vaccine, HEV239, showed 100% efficacy after three doses in phase III trial in a Chinese population. HEV239 is licensed by China's Ministry of Science and Technology,and is being produced and Marketed by Xiamen Innovax Limited (Hoon *et al*.,2014).

The vaccine will presumably only be used in endemic areas,the military or in travelers to high-risk areas results from both animal and human studies have suggested that repeated exposures to virus or viral antigens result in more robust immunogenicity and confer greater protection from overt disease upon subsequent viral challenge.However,subclinical HEV infection subsequent to vaccination has been demonstrated in monkeys in several preclinical trials and more recently confirmed in followup of humans participating in the large recombinant vaccine trial in Jiangsu,China .The duration of protection from clinical disease remains unknown,and optimal dosing and booster schedules remain to be determined. (Krain *et al*.,2014).A recombinant, 3-dose series HEV vaccine is available but has not yet been prequalified by the World Health Organization.The vaccine has been shown to prevent symptomatic HEV infection and proven to be safe and effective in persons aged 16–64 years,safety for children is unknown, the vaccine is expected to be protective against HEV genotype 1 (Thomas *et al*.,2013).

Chapter three
Material and Methods

3.Materials and Methods

3.1 **Study approach**

Qualitative approach

3.2 **Study design**

The study was descriptive ,cross-sectional study

3.3 **Study area**

The study was conducted in Dongola Maternity Hospial,Northren State.

3.4 **Study period**

The study was carried out during November 2015.

3.5 **study population**

Pregnant women in different timestre attending to Dongola Maternity Hospital.

3.6 **Inclusion criteria**

Pregnant women were included in this study.

3.7 **Exclusion criteria**

Non pregnant women were excluded.

3.8 **Data collection**

Data were collected by using a direct interviewing questionnaire(appendix 1)with informed consent.

3.9 **Samle size**

Ninety blood samples were collected from ninety pregnant women

3.10 **sample collection**

Under sterial condition 3ml venous blood sample were collected in plain containers,left to settle for 30 minutes at room temperature to clot and then centrifuged at 3000 rpm for 5 minutes ,transfer the serum to labeled apendove tubes ,serum kept in -20 ^{0}C until used.

3.12 **Ethical Consideration**

Permission of this study was optained from Ministry of Health ,Northren State and College of Graduate Studies of SUST.All individuals participating in the study were informed clearly and simply about the purpose of the study ,and written verbal consent was optained.

3.13 Laboratory methods

3.13.1 ELISA (Enzyme Linked Immunosorbant Assay)-(EUROIMMUN Kits) for detection of HEV antibodies(IgG,IgM).

3.13.1.2principle

IgG

The ELISA test Kit provides a quantitative or semiquantitative in vitro assay for human antibodies of the IgG class against hepatitis E antigens in serum or plasma.The 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 wells.In the case of positive samples ,specific IgGantibodies will bind to antigens.To detect the bound antibodies,second incubation is carried out using an enzyme-labelled anti-human IgG(enzyme conjugate)catalysing a colour reaction.

IgM

The ELISA test kit provides a semiquantitative in vitro assay for human antibodies of IgM class against hepatitis E antigens in serum or plasma .The test kit cantains 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 wells.In the case of positive samples specific IgM antibodies will bind to antigens.To detect the bound antibodies,second

incubation is carried out using an enzyme-labelled anti-human IgM (enzyme conjugate)catalysing a colour reaction.

3.13.1.3 Storage and stability

The test kit has to be stored at a temperature between +2 ^{0}C to +8 ^{0}C .Do not freez.Unopened,all kit components are stable until the indicated expirty date.

3.13.1.4 Test procedure

Preparation of the samples:

The patient samples for analysis are diluted 1:10 with sample buffer (1µl sample to 1.0ml sample buffer) and mix well incubat the mixture for at least 10 minutes at room temperature.subsequently, it can pipetted into micro plate wells according to the pipetting protocol .NOTE:there are two types of sample buffer,one for IgG the second for IgM.

Preparation of the washing buffer:

Warm it to 37 ^{0}C and mix well befor diluted it using distilled water(1 part reagent plus 9 parts distilled water.

Adding samples:

in ELISA microtiter wells add100 µl of calibrater,positive control,negative control and diluted samples in specific order in the well according the protocol of the test.

Incubation of the sample:

The plate was covered and incubated for 30 minute at room temperature(+18 ^{0}C to +25 ^{0}C).

Washing(1):

Empty the wells and subsequently wash 3 times using 300ml of working strenghth wash buffer for each wash,leave the wash buffer in each for 30 to 60seconds per washing cycle ,then emtpty the wells After washing 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.

Addition of conjugate:

Pipette 100µl of enzyme conjugate (peroxidase-labelled anti-human IgG) in to each of the microplate wells.

Conjugate incubation:

The plate was covered and incubate for 30 minutes at room temperature (+18 ^{0}C to +25 ^{0}C).

Washing (2):

Repeat all the steps of washing (1).

Addition of substrate:

Pipette 100µl of chromogen/substrate solution into each microplate wells .

Incubate for 15 minutes at room temperature (+18 ^{0}C to +25 ^{0}C) (protect from direct sunlight).

Stopping the reaction:

Pipette 100µl of stop solution into each of the microplate wells in the same order and the same speed as the chromogen/substrat solution was introduce.

3.13.1.5 Measurment and Interpretation of the results

Photometric measurement of the colour intensity should be made at a wavelength of 450 nm, within 30 minutes of adding the stop solution .

The result evaluated by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator 3.

Interpreting the result as follows:

Ratio <0.8: negative.

Ratio >0.8 to <1.1: borderline.

Ratio >1.1: positive.

Border line:

In case of borderline test result ,an additional patient sample should be taken 7 days later and re- tested in parallel with the first patient sample.the result of both samples allow proper evaluation of titer changes.

For duplicate determinations the mean of two values should be taken .If the tow values deviate substantially from one another the sample should be retested.

3.11 **Data analysis**

All collected data were analyzed using SPSS(Statistical Package of Social Science)program ,Pearson Chi-square test was used to analyze the data .P value <0.05 was considered significant.

Chapter Four
Result

Result

This study was performed during November 2015.Atotal of ninety serum samples were collected from pregnant women attended to Dongola Maternity Hospital ,Northren Sudan with sociodemographic information taken from them serum tested using ELISA procedure for detection HEV antibodies IgG and IgM.13/90(14.44%) were positive to anti-HEV IgG ,while 77/90(85.56%) were negative to HEV-IgG , no positive result of HEV-IgM antibodies were detected (table 1),11(12.2%)with no history of jaundice and have HEV-IgG positive(table 2),7(7.8%) were in age group (26-35),high count among age group have HEV-IgG positive,with significant differences(*p* Value <0.05) (table 3),8 (8.9%) have no history of miscarriage with HEV-IgG positive(table 4),5 (5.6%) with high school education level were HEV-IgG positive (table 5),11 (12.2%) were in urban area with HEV-IgG positive (table 6),8 (8.9%)were in third trimester with HEV-IgG positive (table 7),no significant differences regarding these risk factors(history of jaundice, history of miscarriage,education level,residence and trimester) as show in table (2,4,5,6and 7 respectively (p >0.05).

Table 1:Prevelance of IgG and IgM antibodies using ELISA:

Immunoglobulin	Result: positive	Result: negative	Total
IgG	13(14.44%)	77(85.56%)	90
IgM	0(0%)	90(100%)	90

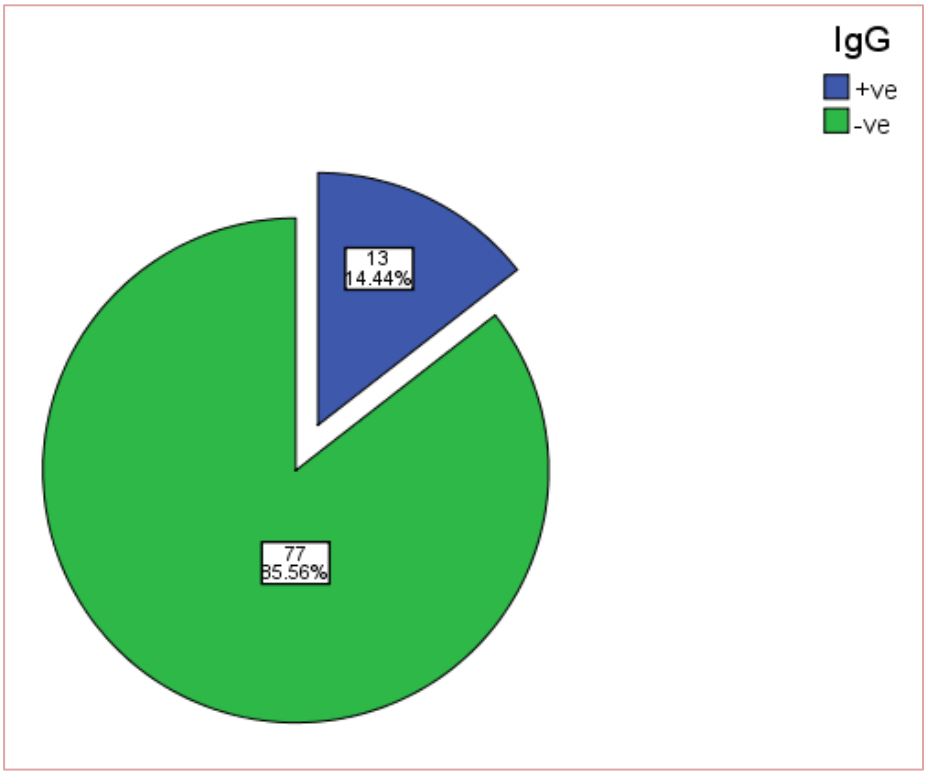

Fig 1:Distribution of HEV-IgG positive.

Table 2:The relationship of the hisrory of jaundice and ELSA, HEV- (IgG).

History of jaundice	IgG positive	IgG negative
Yes	2 2.2%	12 13.3%
No	11 12.2%	65 72.2%

***P* value (0.985).No significant differences at the 0.05 level.**

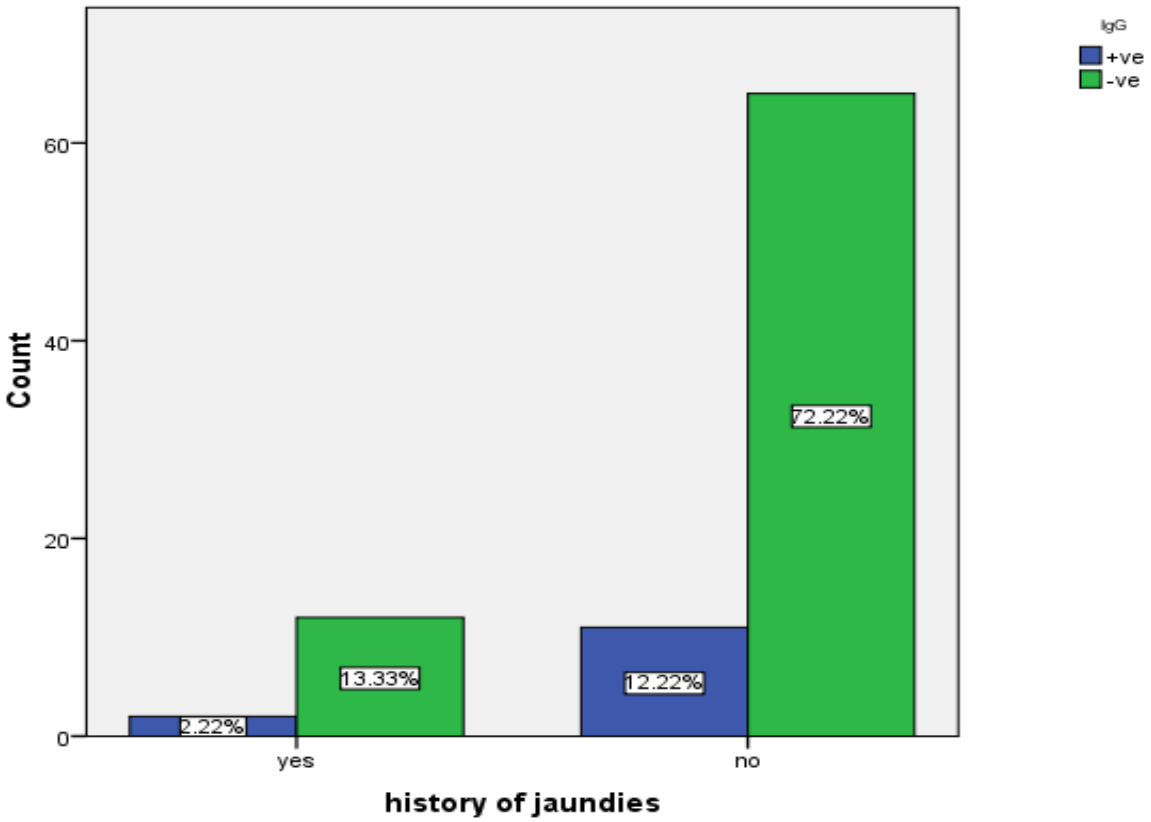

Fig 2: Distripution of HEV-IgG positive according to history of jaundies.

Table 3:The relationship of age group and ELISA-HEV(IgG).

Age group	IgG positive	IgG negative
15-25	2 2.22%	32 35.56%
26-35	10 11.11%	30 33.33%
36-45	1 1.11%	15 16.67%

P value (0.039),there was a significant differences at the 0.05 level.

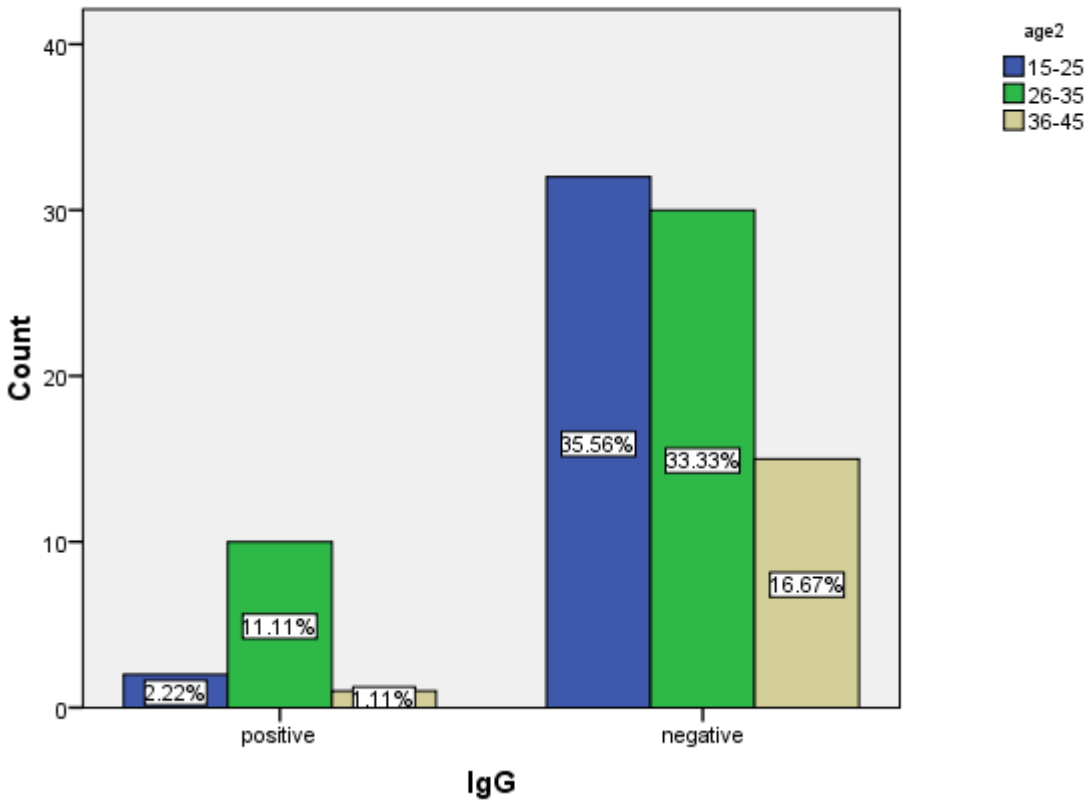

Fig 3:Distribution of HEV-IgG positive according age groups.

Table 4:The relationship of the history of miscarriage and ELISA- HEV(IgG) positive.

History of miscarriae	IgG positive	IgG negative
yes	5 5.6%	26 28.9%
No	8 8.9%	51 56.7%

P value o.742,no significant differencses at o.o5 level.

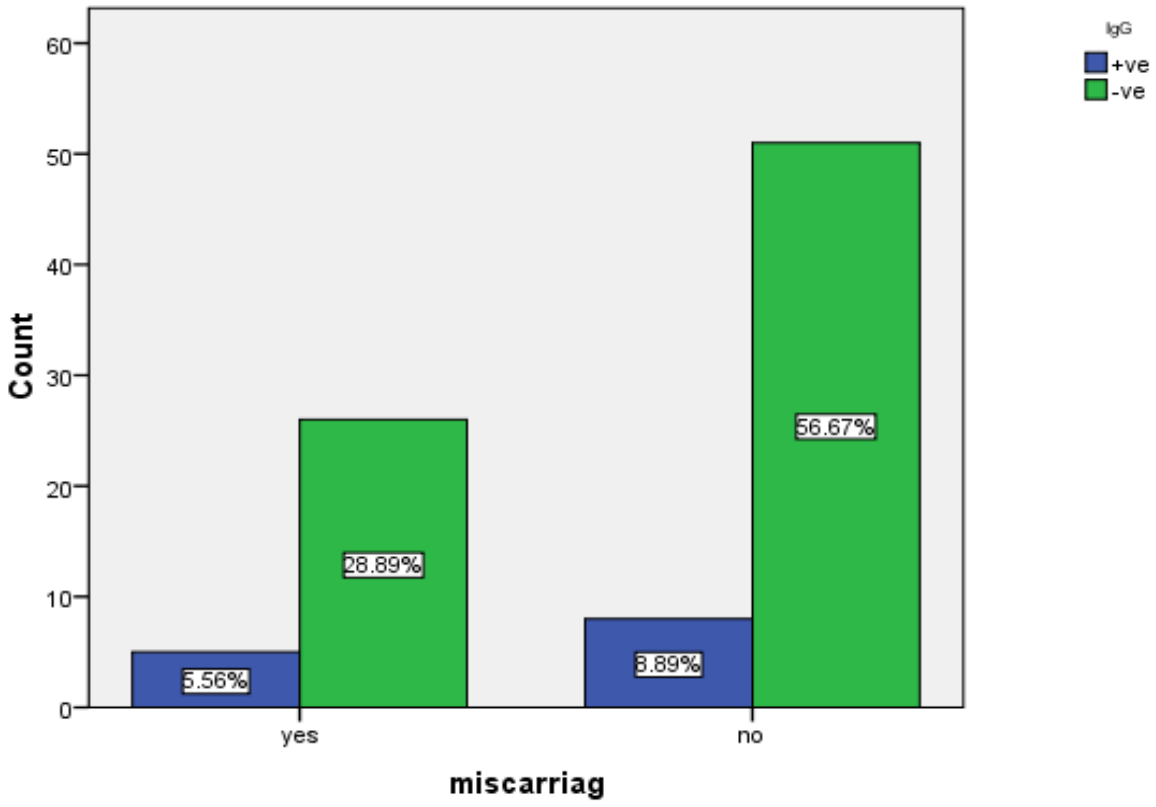

Fig 4:Distribution of HEV-IgG positive according history of misscarriag.

Table 5:The eralationship of education level and ELISA_HEV (IgG).

Education level	IgG positive	IgG negative
Illutrated	1 1.1%	7 7.8%
Primary school	3 3.3%	33 36.7%
Middle school	0 0.0%	1 1.1%
High school	5 5.6%	20 22.2%
University	4 4.4%	16 17.8%

P value 0.662,no significant differences at the o.o5 level.

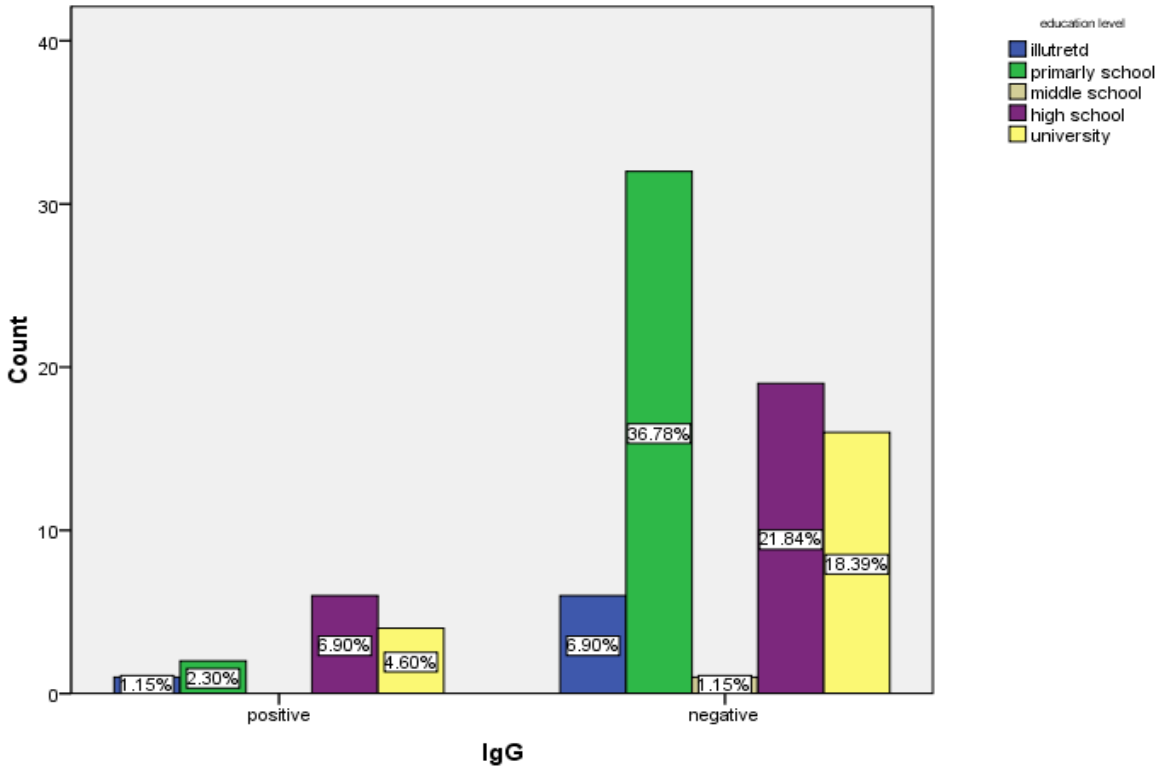

Fig 5:Distribution of HEV-IgG positive according education level.

Table 6:the relationship of the residence and ELISA HEV (IgG).

Residence	IgG positive	IgG negative
Rural	2 2.2%	34 37.8%
urban	11 12.2%	43 47.8%

P value 0.05,no significant differencses at the o.o5 level.

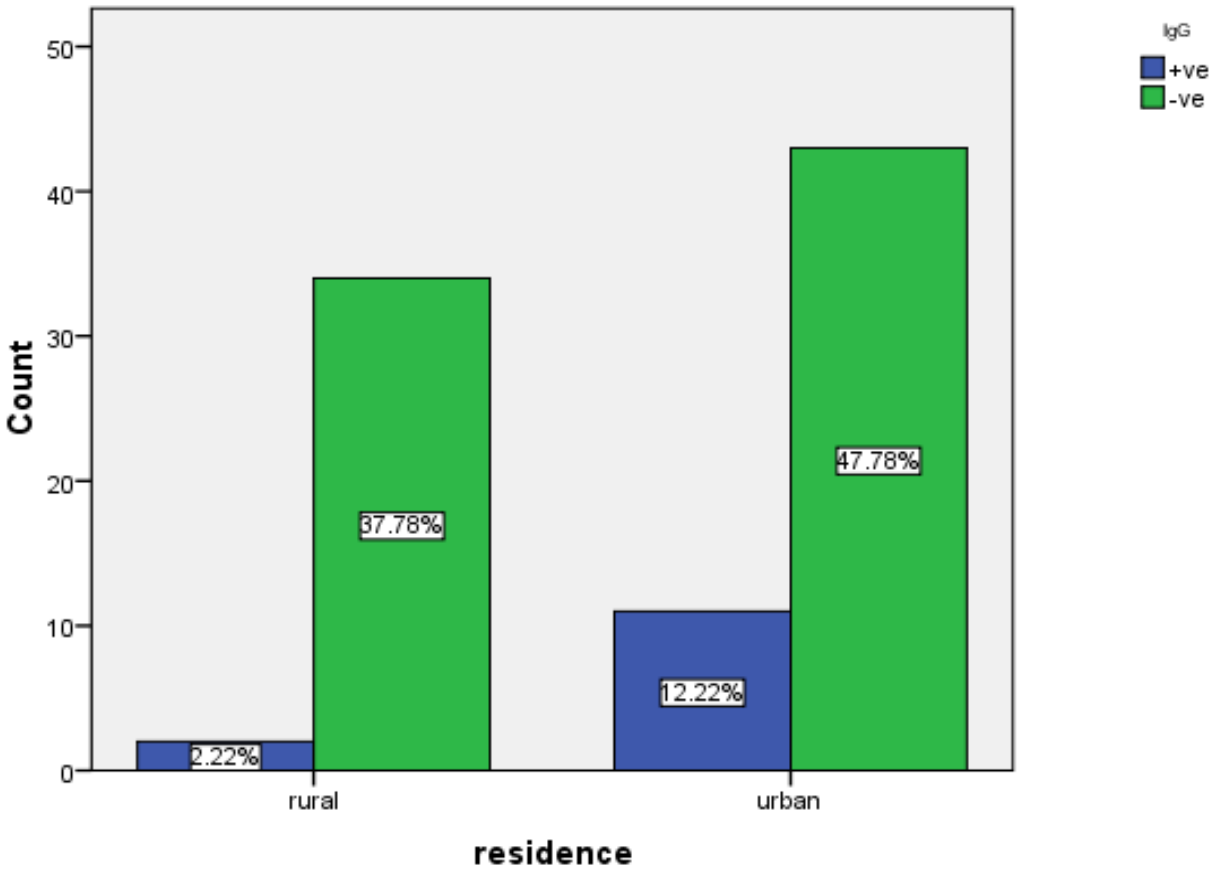

Fig 6:Distribution of HEV-IgG positive according to residence.

Table 7:the relationship of the trimester and ELISA HEV (IgG) .

Trimester	IgG positive	IgG negative
First trimester	3 3.3%	15 16.7%
Second trimester	2 2.2%	17 18.9%
Third trimester	8 8.9%	45 50%

P value 0.850,no significant differences at the 0.05 level.

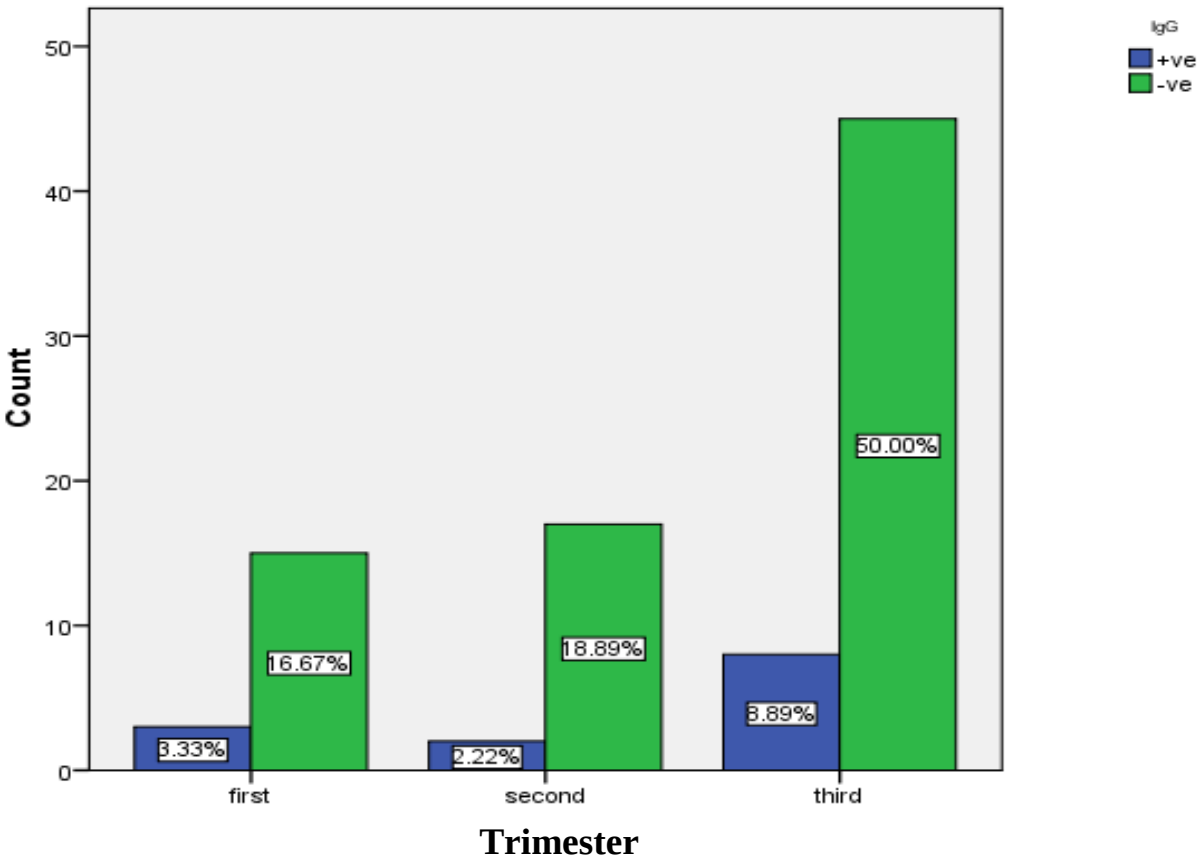

Fig 7:Distripution of HEV-IgG positive according to trimester of pregnant women.

Chapter five
Discussion

Discussion

The main fndings of the current study were: the relatively high prevalence (14.44%) of HEV IgG antibodies among pregnant women regard-less of their age, residence . educational levels,history of jaundice , miscarriage and trimester 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 .In related studies, reported prevalence rates of 84.3% and 60%, among pregnant women in Egypt and India respectively (Boccia *et al* .,2006,stoszek *et al*.,2006,Patra *et al* 2007).Significant relation could be seen between resident of pregnant women and anti HEV antibody positivity demonstrating that highest rate of anti HEV was found in urban area than in rural area this agree with the pregnant women in Gabon ,had anti-HEV IgG and the prevalence was signifcantly higher in the urban areas than in the rural ones (13.5 vs. 6.4%). Also significant relation between age group of pregnant women and anti HEV seropositive,(11.11%)in age between 26-35,that agreed with Adjei *et al*.,(2009)and AL-Tayeb *et al*(2014) that (28.66%)HEV IgG seroprevalence was observed among Ghanaian pregnant women, especially pregnant women who were 21–25 years of age and women in their third trimester .

Conclusion

This study conclude that 13 (14.44%) of anti_HEV IgG antibodies were detected among pregnant women in northern state of sudan , the most age group affected were(26-35) age group were (11.11%),and the third trimester is major compare with other trimester it was (8.9%).

Recommendation

1-.More samples are needed to be collected for disease surveillance and outbreak detection.

A- Increase awareness of HEV infection amongst physicians to enhance its diagnosis and reporting, in order to facilitate early epidemiological investigation and outbreak detection.

B- Enhance the testing for hepatitis E in all acute hepatitis cases,especially those that test negative for hepatitis A and B.

C- Strengthen the use of molecular characterization to determine the local prevalence of different genotypes and to identify clustering of cases.

2.Public health education

- Raise the awareness of general public specially pregnant women about the risks of HE and other enteric infections through various channels

3- Conduct regular review of serological data on hepatitis E to monitor the prevalence of infection and changes in its epidemiology.

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Appendix

Questionnaire

Sudan University of Science and Technology

Collage of Graduate Studies

Microbiology Department

Questionnare

Title:serodetection of HEV antibodies(IgG,IgM)Among pregnant women in Dodola Maternity Hospital,Northren Sudan

By :Hiba Ali Younes

Supervisor :Prof. Yousif Fadllala

Date :…….

Name :…….

Age:……..

History of diagnosis of jaundies?

Yes ☐ no ☐

History of miscarriage?

Yes ☐ no ☐

Where you leave?

Urban area ☐ Rural area ☐

Education level?

Illiterate ☐
Primarly school ☐
Middle school ☐
High school ☐
University ☐

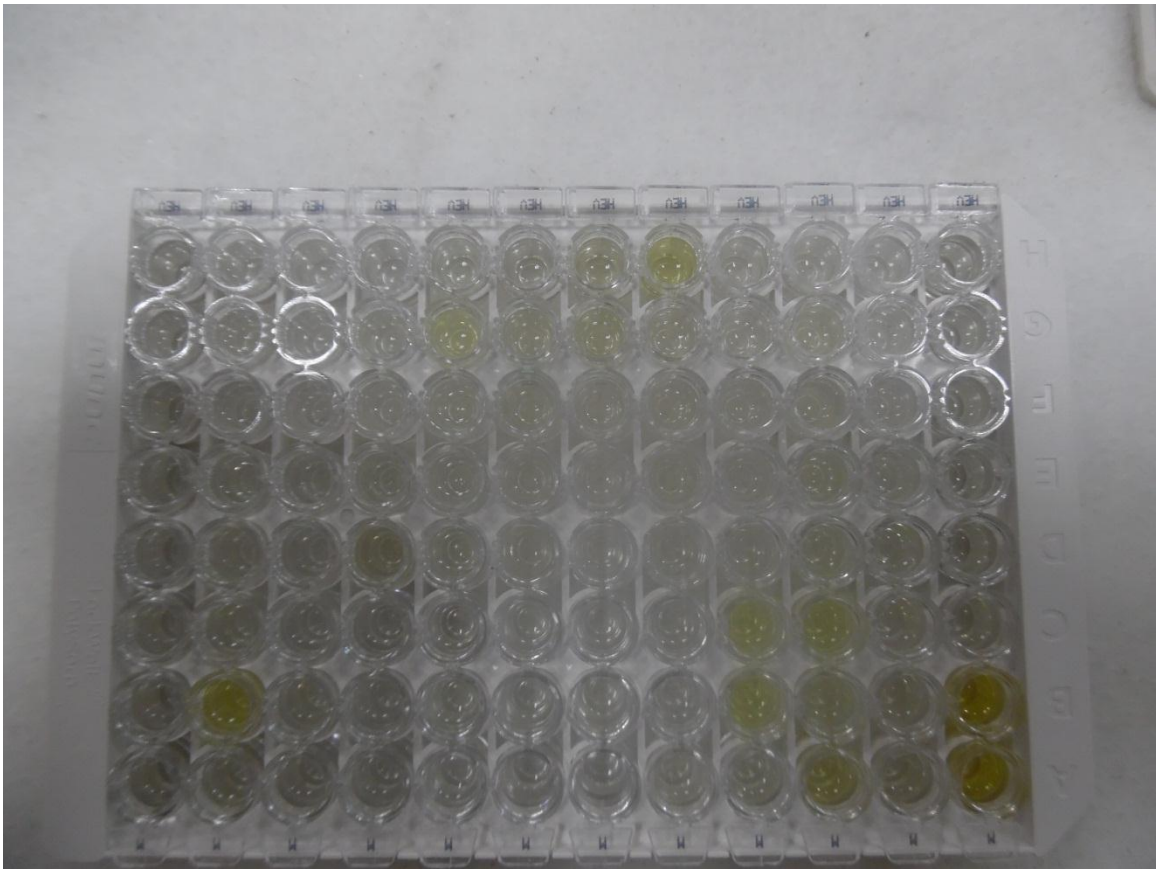
Gestational stage:

First trimester ☐

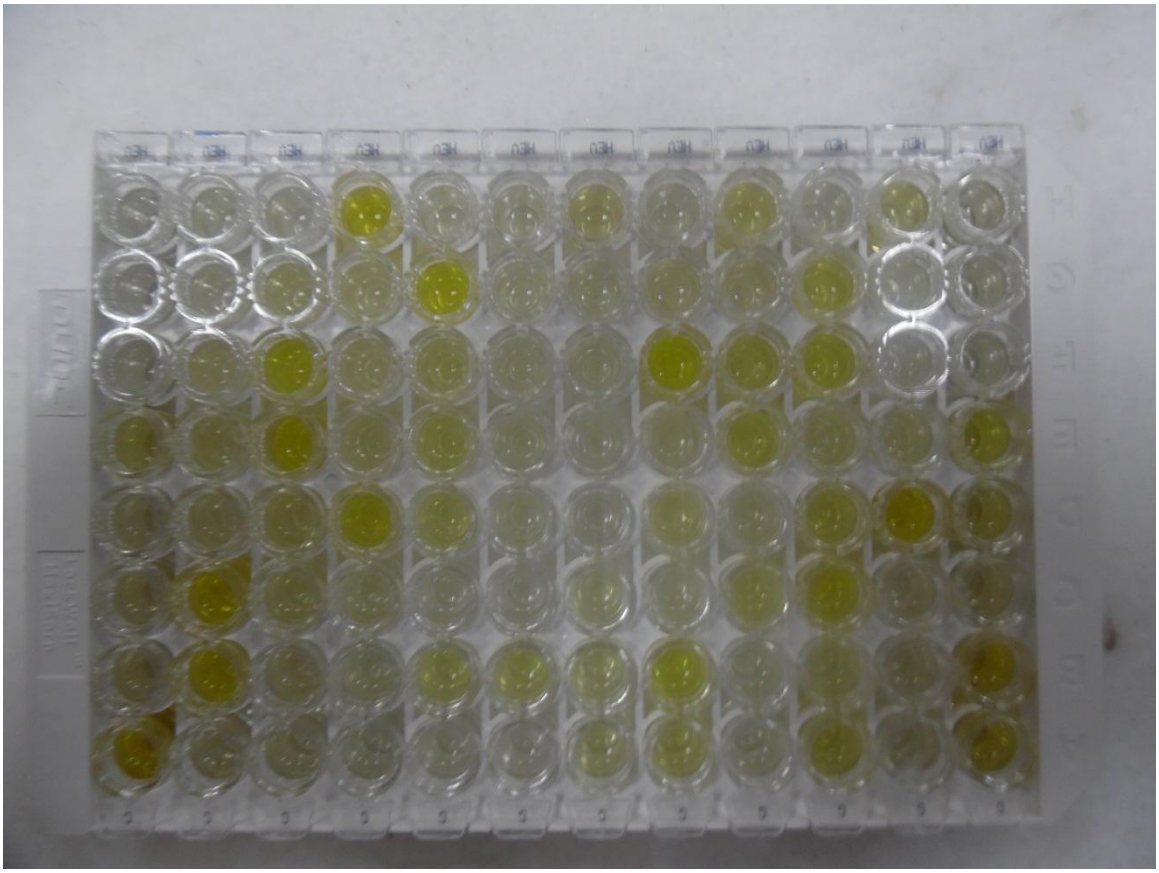
Second trimester ☐

Third trimester ☐

HEV-IgG ELISA kits



Result of HEV-IgM in ELISA microplate



Result of HEV-IgG in ELISA microplate

HEV-IgM ELISA kits