1. INTRODUCTION

1.1 Introduction

The HEV is a spherical, non-enveloped, single-stranded, positive sense RNA virus that is approximately 32 nm to 34 nm in diameter and is the only member in the family Hepeviridae and genus Hepevirus (Teshale and Hu, 2011).

The genomes of several HEV strains from different parts of the world have been sequenced and compared. They can be generally distinguished into four major groups, namely genotype 1, 2, 3 and 4 respectively. All HEV strains appear to comprise a single serotype. No serologic or hybridizing cross-reactivity between HEV and other viral hepatitis agents, including hepatitis A virus (HAV) has been observed (Previsani and Lavanchy, 2001).

Hepatitis E virus (HEV) is a significant international public health problem and it is estimated that 2.3 billion people are infected globally (WHO, 2010). HEV is the leading cause of acute viral hepatitis in the world, especially in developing countries. The first retrospectively confirmed outbreak of hepatitis E occurred in 1955-1956 in New Delhi, India and resulted in more than 29000 symptomatic jaundiced persons (Teshale and Hu, 2011).

HEV is transmitted via the fecal-oral route and rarely through person-to-person transmission. HEV is recognized as a common source of waterborne outbreaks involving fecal contaminated water (Boccia *et al*., 2006).

Hepatitis E is an 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 (Teshale and Hu, 2011).

In Sudan studies on patients with acute hepatitis during the floods of 1988 in Khartoum demonstrated that infection was mainly due HEV 58% with low

incidence 5.45% of HAV infection. Another study amongst children with acute hepatitis in Khartoum state concluded that HEV was also the commonest cause of acute clinical hepatitis among that pediatric population with HEV infection at 59%, HAV at 33.3%, and HBV at 2.6%.The largest documented outbreak of HEV infection in displaced populations was reported from Mornay camp in western Darfur in 2004, when, out of a total population of 78,800 people, 2621 were infected with HEV, with an attack rate of 3.3% and an overall case-fatality rate of 1.7% (Mudawi, 2008).

1.2 Rationale

Hepatitis E virus is probably the most common cause of acute hepatitis and jaundice in the world (Purcell and Emerson, 2008). HEV tends to give rise to more prominent cholestasis and the infection can present as acute fulminating hepatitis. Extra-hepatic manifestations of hepatitis E include arthritis, pancreatitis, and aplastic anemia, as well as such neurologic complications as polyradiculopathy, the Guillain–Barré syndrome, Bell's palsy, peripheral neuropathy, ataxia, and mental confusion (Kamar *et al.*, 2011).

The incidence and prevalence rate of HEV infection among children in Khartoum confused with HAV because the two viruses share certain basic clinical and epidemiological properties and unavailability of laboratory services to differentiation between them.

Due to above facts this is study was designed to determine the incidence of HEV among children.

1.3 Objectives

1.3.1 General objective

To determine the presence of acute hepatitis E viral infection among children in Khartoum State.

1.3.2 Specific objectives

1. To detect the presence of anti-HEV IgM antibodies among children.
2. To identify the major possible risk factors associated with HEV infection (gender, age, and geographic area).

2. Literature review

2.1 Hepatitis E virus

2.1.1 History

Hepatitis E virus (HEV), the etiological agent of hepatitis E (HE), was described for the first time using electron microscopy in 1983 as a spherical viral particle being 27 to 30 nm in size. The virus originated from the stool of a volunteer orally infected with faeces from suspect cases of non-A and non-B hepatitis (Balayan *et al*., 1983). The HEV genome comprises a non-segmented positive-sense RNA chain and the virus is non-enveloped (Acha and Szyfres, 2003). HEV was suggested to be classified in the Picornaviridae family (Balayan *et al.,* 1983). However, later studies showed that it does not belong to members of this family. Between 1988 and 1998, HEV was tentatively classified in the Caliciviridae family, based on virion morphology.

This classification was also rejected after a phylogeny analysis of the HEV genome, and HEV was newly classified as an independent genus HEV-like virus, unassigned to any family (Acha and Szyfres, 2003). At present, HEV is the only member of the Hepevirus genus, Hepeviridae family (Emerson *et al*., 2004).

2.1.2. Classification

HEV belongs to the genus Hepevirus in the family Hepeviridae and consists of four recognized genotypes and at least two putative new genotypes (Meng, 2010) Genotype 1 causes large outbreaks of acute hepatitis E in humans in Asia.

Genotype 2 causes outbreaks in humans and includes one Mexican strain and several African strains. Genotype 3 is associated with sporadic, cluster, and chronic cases of hepatitis E in humans, mostly in industrialized countries. Genotype 3 HEV is known to be zoonotic and has also been isolated from domestic and wild swine, deer, mongoose, rats, and rabbits (Tei *et al*., 2003; De Deus *et al*., 2008).

Genotype 4 HEV is also zoonotic and is associated with sporadic cases of hepatitis E in humans and infects wild and domestic swine and reportedly cattle and sheep (Meng, 2010).

2.1.3 HEV biology

The genome of HEV is a single-stranded, positive-sense, RNA molecule of approximately 7.2 kb in size (Emerson and Purcell, 2003). The genome consists of three open reading frames (ORFs), a 5′ non-coding region (NCR), and a 3′ NCR (Kumar *et al*., 2013).

2.1.4 Routes of transmission

HEV is mainly an enterically transmitted virus that causes waterborne epidemics in developing countries and sporadic cases in developed countries. There are four reported routes of transmission, namely waterborne, zoonotic (foodborne), bloodborne and perinatal (Mushahwar, 2008). Person-to-person transmission of hepatitis E is rare compared with hepatitis A, which might be related to the low amount of

intact HEV particles present in a patient’s stool (Previsani and Lavanchy, 2001).

2.1.5 Risk groups

People who are at risk of contracting HEV:

• Persons residing in areas where extended community outbreaks exist.

• International travelers to regions of the world where HEV is endemic.

• Refugees residing in overcrowded temporary camps following catastrophies, especially in Sudan, Somalia, Kenya and Ethiopia.

• Persons who have chronic liver disease.

• Possibly persons working with non-human primates, pigs, cows, sheep and goats (WHO, 2001)

2.1.6. Epidemiology

Hepatitis E is important enteric infection causing large scale outbreaks in many parts of the world. Since the 1950s, epidemics of enterically transmitted non-A, non-B hepatitis have been frequently documented in the Indian sub-continent. Large epidemics of hepatitis E have been reported in southeast and central Asia, northern and western Africa, and Mexico. In some highly endemic areas, HEV infection also accounts for more than 50% of acute sporadic hepatitis. Infection occurs more frequently in regions with hot climates. The highest prevalence of infection occurs in regions where low standards of sanitation promote the transmission of the

virus. One of the largest waterborne hepatitis E outbreaks occurred in Kanpur city, Uttar Pradesh, India in 1991 where over 79,000 clinical cases were reported.

The source of this outbreak was traced to faecal contamination of drinking water supplied from the river Ganges (Naik *et al.*, 1992).

In parts of Africa (Sudan, Chad, Uganda, Kenya, and Somalia) a number of large hepatitis E outbreaks have occurred among persons living in refugee camps or internally displaced persons camps (Boccia *et al.*, 2006). Persons living in such camps may not have adequate access to clean water and sanitary conditions. Furthermore, such populations may be vulnerable to infectious diseases because of crowded living and poor nutrition, leading to higher risk of exposure to infectious agents and poor immune response during infectious exposures. Available medical care services may not be optimal and thus mortality from serious complications of infection may be high (Teshale and Hu, 2011).

2.1.6.2 Geographic distribution according to genotypes

The genomes of many HEV strains from Asia, South and North America have been entirely or partially sequenced. The human HEV strains have at least four major groups.

Human genotype 1 has been the major cause of water-borne epidemics in Asia and North Africa and a significant cause of sporadic disease, while human genotype 2 has been recovered from epidemics in Mexico and central Africa. Hepatitis E caused by genotypes 3 and 4 more commonly occurs in developed countries and on average affects older people (Purcell and Emerson, 2008).

Figure 1: Distribution of hepatitis E virus infection, (CDC, 2012)

2.1.7 HEV pathogenesis

2.1.7.1 Incubation period

The incubation period has a range of 15–60 days, with a mean of 40 days (Eyasu *et al*., 2010).

2.1.7.2 Virus replication

Although some progress has been made in understanding the replication cycle of HEV with use of animal models, cell culture systems, and infectious complementary DNA (cDNA) clones, most aspects of viral replication in vivo can only be inferred. After ingestion of HEV, the virus may be absorbed directly through the gastrointestinal mucosa into the circulation to reach the liver or after one or more rounds of amplification in enterocytes. However, no evidence has been obtained for replication of HEV at this site, in contrast to HAV for which replication in enterocyte derived Caco2 cells is well established (Blank *et al*., 2000).

HEV is likely to interact with one or more specific receptors/coreceptors leading to penetration and uncoating of the virus, and the input viral RNA then serves as mRNA for PORF1. The PORF1 polyprotein is then cleaved by viral (and perhaps cellular) proteases to yield the mature replicative proteins, but the viral protease activity has not been directly demonstrated. The viral RNA-dependent RNA polymerase (RDRP) then copies the input viral genome to yield negative-strand RNA, which in turn serves as a template for the transcription of further positive-strand RNA molecules, including new genomes and the subgenomic, bicistronic RNA

encoding ORF2 and ORF3 proteins. Genomic RNA assembles together with PORF2, although the precise form involved in particle assembly (fulllength or truncated, glycosylated or nonglycosylated) is unclear. PORF3 may also associate with the virus particle during assembly (Takahashi *et al.*, 2008), although it is dispensable for replication in vitro. The virus is then released from the hepatocyte via unknown mechanisms. Asmall amount of HEV is found in plasma during infection, consistent with release of progeny virus through the basolateral domains of hepatocytes leading to spread through the liver, but most of the virus is likely to be excreted through the biliary system to complete the transmission cycle (Arankalle *et al.*, 1993), consistent with release of virus through the apical domain of hepatocytes. Interestingly, however, HAV shows preferential release through the basolateral domain of hepatocytes in vitro, contrary to the similar expectation of its apical release into the system, which suggests an indirect route for excretion of that virus (Snooks *et al.*, 2008).

2.1.7.3 Clinical features

In humans, HEV causes an acute icteric disease that varies in symptoms from subclinical to fulminant hepatitis (Emerson and Purcell, 2003). The asymptomatic patient typically clears the virus rapidly, while the symptomatic patient experiences clinical signs including anorexia, hepatomegaly, myalgia, jaundice and sometimes abdominal discomfort, nausea,

vomiting, and fever (Meng, 2010). In immunocompromised patients such as organ transplant recipients, lymphoma and leukemia patients, or patients with HIV infection, the course of disease may progress to a chronic state with cirrhosis of the liver and persistence of viral shedding (Kamar *et al*., 2008; Haagsma *et al*., 2008). Of particular concern is the ability for HEV-infected immunocompromised individuals to develop clinical disease well after the initial exposure (Ollier *et al*., 2009 ; Teshale *et al .,* 2010) The typical infection begins with an incubation period of 2 weeks to 2 months and a transient viraemia followed by viral shedding in the feces, disappearance of viraemia with the onset of clinical signs, and regression of viral shedding with potential jaundice setting in around 2–3 weeks into the infection (Haagsma *et al*., 2008).The severity of HEV infection is considered dose-dependent and host factors such as concurrent hepatic disease or alcohol overuse may also contribute to the disease course (Yugo and Meng, 2013).

2.1.8 Laboratory diagnosis

2.1.8.1 Diagnostic assay formats

Based on the analysis of serum specimens collected during various stages of human HEV infection, which include the incubation period, acute and convalescent phases, a classic serological pattern of IgM and IgG anti-HEV appearances has been observed (Khudyakov and Kamili, 2011). IgM anti-HEV appears during the early acute phase of illness and may be

detected as early as 4 days after the onset of jaundice and lasts for up to five months (Khudyakov and Kamili, 2011).

The serological appearance of IgM anti-HEV is succeeded shortly by IgG anti-HEV, so that both seem to appear almost simultaneously in the acute phase of infection. IgG anti-HEV persists for a longer time and may be detectable for 1–14 years (Khudyakov and Kamili, 2011). HEV RNA can be detected in serum and stool of infected patients during the acute phase of infection by RT-PCR using conventional and real-time formats (Mushahwar, 2008).

2.1.8.2 Rapid diagnostic assays

In addition to standard enzyme immunoassays, other assays formats like rapid tests have also been evaluated for HEV serology. The immunochromatographic methods for the detection of serological markers of infections are fast and simple and are frequently formatted into rapid diagnostic assays suitable for point-of-care testing. A rapid immunochromatographic assay ASSURE™ has been developed by Genelabs Diagnostics, Singapore and evaluated for the detection of IgM anti-HEV in serum specimens from patients with acute hepatitis E infection (Khudyakov andKamili, 2011).This test is an IgM-capture lateral-flow fimmunochromatographic assay. In this format, anti-HEV IgM is captured by anti-human IgM monoclonal antibody immobilized onto the membrane and detected using a colloidal gold-labeled HEV antibody attached to HEV antigen.

Rapidity and simplicity to perform are major advantages of the ASSURE™ HEV IgM test. It was recently found to be highly sensitive (82%) and specific (100%) in detection of acute HEV genotype 3 infections (Khudyakov and Kamili, 2011).

2.1.8.3 Immune fluorescence microscopy

A few specialized laboratories use this technique for the detection of antibodies. IFE detects antibodies that react against the HEV antigen semiquantitatively. Anti-HEV antibodies block the binding of fluorescein-conjugated anti-HEV IgG to HEV antigen in frozen liver tissue. The concentration of anti-HEV antibodies is estimated semiquantitatively. This method is laborious and expensive and thus not useful for routine diagnosis (Vasickova *et al.*, 2007).

2.1.8.4 Indirect ELISA

Indirect ELISA is one of the most popular diagnostic formats. In this format, HEV-specific immunoglobulins captured from serum specimens by HEV antigens attached to the solid-phase surface (usually wells of microtiter plates) are detected using labeled species-specific antibody. The differential detection of IgG or IgM is achieved using, for example, antibody against gamma-or mu-chains of antibodies, correspondingly. There are numerous examples of application of this format to the detection of IgG anti-HEV in serum specimens using *E. coli*-expressed HEV antigens (Obriadina *et*

al., 2002) and virus-like particles expressed in insect cells (Khudyakov and Kamili, 2011).

2.1.8.5 Sandwich ELISA

HEV genotypes 3 and 4 infect human and swine hosts (Meng, 2010). The detection of anti-HEV in humans or swine requires assays designed specifically for specimens from humans or animals. The host-independent detection of acute or past HEV infections has, however, significant advantages for epidemiological investigations in the field. The format that allows for such host-independent detection is the double-antigen sandwich ELISA. In this format, antigen attached to solid phase is used to capture specific antibody from serum specimens. Detection of this antibody is achieved using the same antigen labeled with, for example, horseradish (Khudyakov and Kamili, 2011).

2.1.8.6 Western blot-based assay

HEV recombinant proteins have been used in different formats of diagnostic assays. A Western blot-based assay for the detection of HEV IgG and IgM antibodies was developed using a recombinant polypeptide containing the C-terminal half of the ORF2-encoded protein (opening reading frame2-encoded protein) (Khudyakov and Kamili, 2011). Although cumber-some to perform, this assay could detect IgG anti-HEV in 89–100% of non-A, non-C patients 1–24 months after onset of jaundice and IgM in 73% of patients within 26 days after onset of jaundice. Another Western blot assay based on a

different set of fusion proteins containing sequences from ORF2 and ORF3 of the HEV genotype 1 strain was used to examine temporal appearance of HEV antibodies in experimentally infected rhesus monkeys (Khudyakov and Kamili, 2011).

An assay based on this format was recently developed for detection of anti-HEV in human and animal specimens (Hu *et al.*, 2008). This assay showed the specificity of 98.8% with human samples. It could detect specific antibody in experimentally infected pigs 14 days after inoculation. Another very important feature of this assay is that it does not discriminate between classes of antibody, thus detecting total anti-HEV (Khudyakov and Kamili, 2011).

2.1.8.7 Molecular techniques

Nucleic acid-based techniques, especially nested real-time PCR (RT-PCR) and real-time RT-PCR, have emerged rapidly as the method of first choice for sensitive and specific detection of RNA viruses (Vasickova *et al.*, 2007). This molecular method consists of two or three steps (nested RT-PCR). The first step of RT-PCR, reverse transcription, uses specific primers, random hexamers or Oligo dT to rewrite viral RNA into cDNA. In the second or third step, PCR or nested PCR uses specific primers to amplify specific segments of viral RNA. Primer binding sites can be spread over the whole genome. However, nested RT-PCR is prone to contamination and virus quantification cannot be undertaken. To overcome

these difficulties, rapid and sensitive real-time RT-PCR assays have been developed for the detection of HEV RNA in clinical samples (Ahn *et al*., 2006; Enouf *et al*., 2006).

2.1.9 Treatment of HEV infection

Most cases of acute HEV infection are self-limiting and require no treatment. However, patients with or without pre-existing chronic liver disease with acute severe HEV infection, have been treated successfully with ribavirin monotherapy (Peron *et al*., 2011; Gerolami *et al*., 2011).

2.1.10 Prevention of hepatitis E virus infection

Two subunit vaccines have been developed against HEV infection and have been shown to be highly protective against clinical hepatitis E in clinical trials (Shrestha *et al*., 2007; Zhu *et al*., 2010). Currently only one of these vaccines has been licensed and that too only in China. Though several additional pieces of information are needed before these vaccines can be used for prevention of hepatitis E in general population,. In the meanwhile, our knowledge about epidemiology of hepatitis E allows us to devise and use non-vaccine approaches for prevention of this disease. In high endemicity areas, these primarily include provision of safe drinking water, proper disposal of human feces, and education about personal hygiene. In addition, during outbreaks, boiling and

chlorination of water should be useful. In contrast, in low-endemicity areas with zoonotic transmission, sanitary handling and proper cooking of pig and deer meat may be important (Aggarwal, 2013).

2.2 Background studies

In 1992 in Sudan using a Western blot assay showed that 23 (59%) of 39 Sudanese children (age range 2-14 years; mean 6.5) with acute viral hepatitis had IgM antibodies to HEV-specific peptides in their sera, sixteen of the 23 children positive for IgM anti-HEV also had IgG anti-HEV antibodies. Among 39 concurrently studied controls, seven had IgG anti-HEV and three others had IgM anti-HEV. These findings suggested that HEV was the most common cause of acute sporadic hepatitis among Sudanese urban children (Hyams *et al*., 1992a).

In Egypt estimated the prevalence of anti-HEV IgG and IgM antibodies to ORF3 peptide of Hepatitis E virus genome in an age of children, study subjects (100 children) between 6 months and 10 years with minor, hepatic illnesses were recruited for the study during the period from September 2004 to September 2005. Serum anti-HEV IgG and anti-HEV IgM antibodies were screened in all subjects. Out of 100 subjects recruited, 26 subjects (26%) demonstrated anti-HEV IgG and 6 (6 %) were anti-HEV IgM and IgG positive. Serum transaminases were raised in one (17%) of subjects with anti-HEV IgM antibodies. The study concluded that children are

susceptible to HEV infection since early infancy. Seropositivity to HEV antibodies increased by over 2 times beyond 4 years of age when compared to younger age (Aboulata *et al.*, 2005).

In 2013 in Turkey anti-HEV antibodies were investigated in 185 primary school children (91 from rural areas and 94 from urban areas of Denizli). The children were divided into two age groups as seven-year old group and fourteen-year old group. Samples were tested for anti-HEV Ab by an enzyme-linked immunoassay. A total of 23 primary school children were anti-HEV Ab positive, giving a prevalence of 12.4%. The seroprevalence rate was 13.1% in rural areas and 11.7% in urban areas. The difference in the seropositive rates was not statistically significant ($p>0.05$). Among 185 primary school children, Anti-HEV antibodies were positive 17 (18.1%) in seven-year old group, and 6 (6.6%) in fourteen-year old group. The difference in the seropositivity rates was statistically significant ($p<0.05$). The study suggested there was no association between the anti-HEV Ab and gender, socioeconomic level, parental educational level, rural or urban areas. Anti-HEV Ab seroprevalence was higher in seven-year old children than fourteen-year old children (Cevahir *et al.*, 2013).

3. Materials and Methods

3.1 Study approach

Qualitative approach

3.2 Study type

The study was population laboratory-based study.

3.3 Study design

This study was cross-sectional study.

3.4 Study area

This study was conducted in Alboluk Pediatrics Hospital.

3.5 Study period

This study was carried out in the period from October 2013 to January 2014.

3.6 Study population

Children age ranged between 2-14 years attending Alboluk Pediatrics Hospital.

3.7 Inclusion criteria

Children with signs and symptoms of jaundice were included in this study.

3.8 Exclusion criteria

Children without signs and symptoms of jaundice were excluded.

3.9 Sampling technique

Simple random clustering technique.

3.10 Sample size

91 blood samples were collected from 91 children.

3.11 Data analysis

Collected data were analyzed by a computer system using statistical package for social science (SPSS) program using the Chi square test and crosstabulation. Statistical significant was set at *p*-values < 0.05.

3.12 Ethical Consideration

Permission from parents was taken and they were aware with the study and the results obtained. The study protocol was reviewed and passed by the Ethical and Scientific Committees of SUST.

3.13 Experimental work

3.13.1Method of data collection

Data was collected through an interviewed Questionnaire (appendix).

3.13.2 Specimen Collection

Under sterile condition, 3ml of venous blood were collected in sterile plain containers and allowed to clot at room temperature. The sera were obtained by centrifugation of the blood at 3000 rpm for 5 minutes. The serum was separated from the clot and transferred into new sterile labeled plain containers and stored at -20^{o}C until used.

3.13.3 Laboratory methods

3.13.3.1 ELISA technique for detection of HEV

Enzyme-Linked Immunosorbent Assay (ELISA) was used to detect anti-HEV IgM antibody (Wantai, China).

3.13.3.2 Principle

This is an ELISA assay for qualitative determination of IgM-class antibodies to hepatitis E virus in human serum or plasma samples. The assay is intended to be used in clinical laboratories for diagnosis and management of patients to infection with hepatitis E virus.

A solid phase antibody capture ELISA assay in which polystyrene microwell strips are pre-coated with antibodies directed to human immunoglobulin M proteins. The patients' serum/plasma samples added, and during the first incubation step, any IgM class antibodies will be captured in the well. After washing out all the other substances of the sample, the specific HEV IgM captured on the solid phase is detected by the addition of recombinant HEV ORF2 antigen conjugated to enzyme horseradish peroxidase (HRP-conjugate). During second incubation, the HRP-conjugated antigens will specifically react only with HEV IgM antibodies. After washing to remove the unbound HRP-conjugate, chromogen solutions are added into the wells. In presence of (anti-HEV-IgM) – (HEVAg-HRP) immunocomplex, the colorless chromogens are hydrolyzed by the bound HRP-conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the amount of antibody in the

sample respectively wells containing negative for HEV remain colorless.

3.13.3.3 Assay procedure

Reagents preparation

The reagents and samples were allowed to reach room temperature (18-30ºC) for 15 minutes. The stock wash buffer had been diluted 1 to 20 with distilled water.

Numbering the wells

The strips needed were set in strip holder and sufficient number of wells including one blank (A1), three negative controls (N1, N2, N3) and one control positive (P1) were numbered.

Adding diluents

An amount of 100µl of diluents were added into each well except the blank.

Adding samples

Also10µl of samples and positive and negative controls were added into their respective wells.

Incubation of samples

The plate was covered and incubated for 30 minutes at 37ºC.

Washing (1)

The plate cover was removed and discarded. The wells were washed with diluted washing buffer 5 times using ELISA washer (see appendix).

Adding HRP-conjugate

An amount of 100µl of HRP-conjugate were added into each well except the blank.

Incubation HRP-conjugate

The plate was covered and incubated for 30 min at 37°C.

Washing (2)

The plate cover was removed and discarded. The wells were washed with diluted washing buffer 5 times using ELISA washer.

Coloring

An amount of 50µl of chromogen A (urea peroxide soluation) and 50µl of chromogen B (TMB solution) were added into each well including blank. The plate was incubated at 37°C for 15 minutes. Blue color was seen in the positive control.

Stopping the reaction

An amount of 50µl of stop solution were added into each well. The blue color of positive control turned yellow.

Measuring the absorbance

The absorbance was read at 450nm using ELISA reader (see appendix).

3.13.3.4 Quality control, calculation and interpretation of results

Calculation of cut-off value:

Cut-off value = Nc + 0.26 (Nc = the mean absorbance value for three negative controls).

Quality control validation

According to ELISA kit manufacture

The optical density of blank should be less than 0.08 at 450nm.

The optical density of positive control must be equal to or more than 0.8 at 450nm

The optical density of negative controls must be less 0.100 at 450nm.

Interpretation of results:

Negative results:

Samples gave value less than cut-off value were negative for this assay, which indicates that no HEV IgM-class antibodies have been detected therefore there are no serological indications for current infection with HEV.

Positive result:

Samples gave value equal to or greater than cut-off value were considered initially reactive, which indicates that IgM–class antibodies to hepatitis E virus have been detected.

Borderline:

Samples with value to cut-off ratio between 0.9 and 1.1 were considered borderline and resulting of these specimens in duplicates is required to confirm the initial results.

4. Results

The study was carried out during the period from October 2013 to January 2014. Ninety one children with signs and symptoms of jaundice attended to Alboluk Pediatrics Hospital were enrolled in this study.

4.1 Frequency of anti-HEV IgM among children

It was found that 3 (3.3%) were positive for anti-HEV IgM while 88 (96.7%) were negative for anti-HEV IgM (Table 1).

Table (1): Frequency of anti-HEV IgM among children

HEV IgM	Frequency	Percent
Positive	3	3.3
Negative	88	96.7
Total	91	100

4.2 Distribution of anti-HEV IgM according to gender

According to gender 52(57.1%) were males while 39(42.9%) were females. Out of 3 IgM positive of children it was found that 2 males (2.2%) and one female (1.1%) were anti-HEV IgM positive. The gender of the children had no significant effect ($p > 0.05$) on prevalence of anti-HEV IgM among children (Table 2).

Table (2): Distribution of anti-HEV IgM according to gender

HEV IgM Gender	Positive (Percentage %)	Negative (Percentage %)	Total (Percentage %)
Male	2 (2.2%)	50 (54.9%)	52 (57.1%)
Female	1 (1.1%)	38 (41.8%)	39 (42.9%)
Total	3 (3.3%)	88 (96.7%)	91(100%)

(P value= 0.735)

4.3 Distribution of anti-HEV IgM according to geographic area

According to geographic area 41(45.1%) were localized in rural area while 50(54.9%) were localized in urban area. It was found that 2 from rural area (2.2%) and one from urban area (1.1%) were anti-HEV IgM positive the geographic area of the children had no significant effect on prevalence of anti-HEV IgM ($p > 0.05$) show(Table 3)

Table (3): Distribution of anti-HEV IgM according to geographic area

HEV IgM Area	Positive (Percentage %)	Negative (Percentage %)	Total (Percentage %)
Rural	2 (2.2%)	39 (42.9%)	41 (45%)
Urban	1(1.1%)	49 (53.8%)	50 (55%)
Total	3 (3.3%)	88 (96.7%)	91 (100%)

(P value= 0.444

4.4 Distribution of anti-HEV IgM according to age group

According to age group 33 (36.3%) were aged between 2-5 years, 36(39.6%) were aged between 6-10 years while 22(24.2%) were aged between 11-14 years. It was found that all positive anti-HEV IgM were in the group aged between 11-14. The age group had a significant effect on prevalence of anti-HEV IgM ($p < 0.05$) show (Table 4).

Table (4): Distribution of anti-HEV IgM according to age group

HEV IgM Age group	Positive (Percentage %)	Negative (Percentage %)	Total (Percentage %)

2-4	0 (0%)	33 (36.3%)	33 (36.3%)
5-10	0 (0%)	36 (39.6%)	36 (39.6%)
11-14	3 (3.3%)	19 (20.8%)	22 (24.1%)
Total	3 (3.3%)	88 (96.7%)	91 (100%)

(*P* value= 0.008)

5. Discussion

Hepatitis E virus (HEV) is the leading cause of acute viral hepatitis in the world, especially in developing countries (Teshale and Hu, 2011). HEV is recognized as a common source of waterborne outbreaks involving fecal contaminated water (Boccia *et al*., 2006).

The present study revealed the low percentage of anti-HEV IgM (3.3%), among icteric phase children, this study is in agreement with an Egyptian study which revealed that 4% of children with minor hepatic disorder were positive for anti-HEV IgM (Aboulata *et al.,* 2005).

This study was in disagreement with a Sudanese study that revealed that 59% of children had IgM antibodies to HEV-specific peptides in their sera (Hyams *et al.,* 1992a),from that it was observed that HEV infection was reduced in Sudan comparable to last study carried by Hyams (1992a) and this

might be attributed to increase awareness in community. Also disagreement with Egyptian study that revealed that 12% of children had acute HEV infection (Hyam *et al.,* 1992b) and another Egyptian study revealed that 17.2% had acute HEV infection (El-Sayed *et al*., 2006). This variation in result may be due to overcrowding, poor sanitation and contamination of water supply in Egypt. Disagreement also with Indian study revealed that 42.9% of children had acute HEV infection (Mathur *et al.,* 2001). In India high frequency variation because it is high endemic area

Males were higher prevalence than females that there was no significant relation between anti-HEV IgM and gender, that in agreement with Cevahir *et al* (2013).

The geographic distribution is insignificant with prevalence (p>0.05). Amongst 41 rural children, anti-HEV IgM antibodies were present in 2 children, among 50 urban children, anti-HEV IgM antibodies were present in only one child, That in agreement with Cevahir *et al* (2013) who stated that no association of HEV infection in rural and urban area, but it was in disagreement with a Egyptian study revealed that acute HEV infection was significantly higher in rural than urban area (Divizia *et al*., 1999). These differences may be due too high poor water supply system, poor wage disposal, exposure to wild animals and other environmental condition in the rural areas of Egypt comparison to Sudan might have played a role in their infection with HEV.

All infected children were in age group between 11-14 years that has a significant relation ($p<0.05$), this could be due to children more than 10 years uncontrollable and it can drink and eat from contaminated source. That in agreement with Mathur *et al.,* (2001) and Aboulata *et al.,* (2005) who stated that seropositivity to HEV antibodies increased by over 2 times beyond 4 years of age as compared to younger age.

Conclusion

The present study concluded that 3.3% anti-HEV IgM were detected among icteric phase children in Khartoum State. There was no association between HEV IgM antibody and gender, rural or urban areas. Anti-HEV IgM antibody seroprevalence was higher in age group (11to14) than other age groups of children.

Recommendations

1. Enhance the testing for Hepatitis E virus in all acute hepatitis cases, especially those that negative for Hepatitis B and C.
2. HEV vaccine is recommended for risk group.
3. Good personal hygiene, high quality public water supplies, proper disposal of sanitary waste and general food safety should de recommended for prevent HEV infection to be spread.
4. Increase awareness of HEV infection amongst physicians to enhance its diagnosis and reporting, in order to facilitate early epidemiological investigation and outbreak detection.

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Appendix

Questionnaire

1. Gender: Male ☐ Female ☐
2. Age
3. Geographic area: Urban ☐ Rural ☐

4. Signs and symptoms of jaundice:

Dark urine ☐

Yellow color of eyes ☐

Nausea ☐

Vomiting ☐

Fever ☐

5. Previous exposure to jaundice in family: Yes ☐ No ☐
6. If yes: date of exposure

HEV ELISA microplate

HEV-IgM ELISA

ELISA washer

ELISA reader