1. Introduction

1.1 Introduction:

Hepatitis E, previously known as enterically transmitted non-A non-B hepatitis, is a self-limited infection with clinical and morphological features of acute viral hepatitis.The disease, first documented during an epidemic of viral hepatitis in 1955-56 in India was not recognized as a distinct clinical entity until 1980, when sera from affected patients were shown to lack serological markers of acute hepatitis due to A and B viruses (Stefanidis *et al*., 2003).

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 *et al.,* 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) . Mitsui recently reported that hemodialysis patients in Japan were infected with a genotype 3 HEV by blood transfusion *(* Mitsui *et al*.,2004).

A high prevalence of anti-HEV antibody in patients undergoing chronic HD (10.9%), reported in an early study, led to the hypothesis that the oral–fecal may not be the only route of transmission of HEV in this setting (Stefanidis *et al*., 2003).

Hepatitis E occurs primarily in adults, with the highest rates of symptomatic disease being reported in young to middle-aged adults. Although hepatitis E infection is also frequently seen in children, most of them are asymptomatic and do not have signs of jaundice (Aggarwal *et al*., 1997).

There are at least four genotypes of the virus,genotypes 1 and 2 are limited to humans only, and genotype 3 and 4 have animals as their -reservoir and therefore are zoonotic infections(Kudesia and Wreghitt , 2009).

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).

Regarding the severity of illness with respect to the genotype, it has been found that genotype 4 infection tends to cause more severe clinical disease than genotype 3 infection. In a Japanese study comparing the clinical features of an acute infection of hepatitis E by genotype 3 and 4, it was shown that genotype 4 had a higher peak alanine aminotransaminase levels and lower prothrombin time (Ohnishi *et al*., 2006).

HEV infection is responsible for over 50% of acute hepatitis cases, and the blood transfusion route has emerged as possible mean of sporadic HEV infection(Zhuang *et al*., 2014).

The high risk groups for HEV infection include persons who have chronic liver disease, travelers to endemic areas, those residing in areas where extended community outbreaks exist, and persons working with animals such as pigs, cows, sheep and goats from which they may be infected(Previsani and Lavanchy, 2001).

Haemodialysis patients are particularly predisposed to infections .It seems that HD procedure per se as well as disturbance in both innate and adaptive immunity significantly contribute to this susceptibility . Infections are the major cause of morbidity and the second cause of death following cardiovascular events in HD patients (Eleftheriadis *et al*., 2011).

1.2 Rationale

HEV is probably the most common cause of liver diseases (Levinson, 2012).
Although the fecal-oral was considered as the primary infection route , there is controversial evidence for increased risk of the infection and consequent problems in patients on maintenance hemodialysis (Omid *et al*., 2013).

Patients with chronic haemodialysis are always at risk of infectious diseases due to their compromised immune system; Moreover, these patients are at the frequent exposure to infectious agents during their visit to haemodialysis centers. Among viral infections the association of HEV with the parenteral transmission of the virus remains highly controversial (Omid *et al*., 2013).

Hepatitis E virus infection is more prevalent in patients on heamodialysis compared with non haemodialysis control group (Haffar *et al.,* 2017).

So detection of HEV and may gain insight into the possible blood-borne transmission of HEV among haemodialysis patients in khartoum hospitals.

1.3. Objective

1.3.1 General objective:

To determine seroprevalence of HEV among haemodialysis patients undergoing haemodialysis using ELISA technique during October 2016 to February 2017 - Khartoum.

1.3.2 Specific objectives:

1. To detect anti-hepatitis E IgG antibodies in the sera of haemodialysis patients and normal individuals.

2. To detect relation between HEV and haemodialysis.

3. To detect relation between seropositivity of HEV and different factors like age , gender , and duration of haemodialysis.

2- Literature Review:

2.1 Hepatitis E virus

Hepatitis E virus (HEV) is etiological agent of acute hepatitis, it is a non-enveloped, positive sense, single stranded RNA virus. Originally classified with the family of calicivirus but now classified as sole member of genus hepevirus in the family hepeviridae (Tadesse *et al.*, 2013).

HEV is a small (27–34 nm) nonenveloped virus. The viral genome consists of a single-stranded, positive-sense RNA molecule organized into three open reading frames (ORF1, ORF2, and ORF3). ORF1 is involved in viral replication and protein processing through RNA-dependent RNA polymerase. ORF2 encodes the viral capsid protein, which is involved in attachment to host cells and induction of neutralizing antibodies. Finally, ORF3 encodes for a small immunogenic phosphorylated protein (pORF3) involved in virion morphogenesis and release (Arends *et al.*, 2014).

2.2. Classification of hepatitis E virus

There is only one serotype of the virus and classification is based on the nucleotide sequences of the genome (Lu *and Li .,* 2006). Genotype 1 has been classified into five subtypes, genotype 2 into two subtypes and genotypes 3 and 4 have been into ten and seven subtypes respectively . Differences have been noted between the different genotypes. For genotype 1, the age at which incidence peaks is between 15 and 35 years and mortality is about 1%. Genotype 3 and 4—the most common in Japan—are more common in people older than 60 years and the mortality is between 5 and 10% (Vidyashankar *et al.*, 2010).

2.3. Transmission of hepatitis E virus

Hepatitis E virus is major cause of enterically non a ,b hepatitis in many developing countries when sensation is suboptimal, transmission of HEV is generally via the fecal-oral route, person-to-person transmission, and transmission via the parenteral route or Blood borne, transmission of HEV had been investigated as indirect evidence implicating HEV as a potential transfusion risk by many investigators worldwide (Taremi *et al.*, 2007).

2.4. Distribution of hepatitis E virus

Genotype 1 has been isolated from tropical and several subtropical countries in Asia and Africa. Genotype 2 has been isolated from Mexico, Nigeria, and Chad. Genotype 3 has been isolated almost worldwide including Asia, Europe, Oceania, North and South America. Genotype 4 appears to be limited exclusively in Asia . Genotypes 1 and 2 are restricted to humans and often associated with large outbreaks and epidemics in developing countries with poor sanitation conditions. Genotypes 3 and 4 infect humans, pigs and other animal species and have been responsible for sporadic cases of hepatitis E in both developing and industrialized countries (DeMuro *et al*., 2013).

In the United Kingdom the Department for Environment, Food and Rural Affairs (DEFRA) said that the number of human hepatitis E cases increased by 39 % between 2011 and 2012 (DeMuro *et al*., 2013).

2.5. Pathogenesis of hepatitis E virus

In monkeys, viral replication apparently causes liver damage . The immune response successfully eliminates viremia and shedding of virus in feces, while not inducing much damage to the liver. Seroconversion marks the clearing of virus from feces and blood and is correlated with resolution of disease (Previsani *and Lavanchy* ., 2001).

  Although limited, findings from non human primate and human volunteer studies have elucidated aspects of hepatitis E pathogenesis and pathophysiology. Enterically transmitted virus enters the host via the oral route and begins replicating in the intestinal tract. Presumably, the portal veins transport the virus to the liver where it replicates in the cytoplasm of hepatocyes and induces histologic changes (Knipe *et al*., 2001).

Characteristic tissue alterations include focal necrosis throughout the hexagonal liver lobules as well as inflammation and accumulation of phagocytoic Kupffer cells and polymorphonuclear leukocytes. As seen in other viral hepatitis infections, cholestatic hepatitis suppression of biliary secretion is another indicator of HEV. Ballooning of hepatocytes may occur, and some epidemics present an unusual pseudo-glandular reorganization of hepatocytes (Knipe *et al*., 2001).

2.6 Clinical features of hepatitis E virus

2.6.1 Symptoms and signs

The incubation period is approximately 6 (2–9) weeks. The disease may start with general symptoms, like fever, malaise, abdominal discomfort, but the regularity of such a prodromal phase has not yet been convincingly demonstrated. The main symptoms are those of a self-limiting acute jaundice ,usually accompanied by general symptoms. In typical cases the urine becomes dark, the stools pale and there is an enlargement of the liver together with abnormal liver function tests. The development can be rather dramatic, especially among pregnant women. A 10–20% mortality rate in this group has been reported. The reason for this is not known. Chronic liver disease or persistent viraemia has not been observed (Kudesia and Wreghitt, 2009).

Differential diagnosis. It is not possible to differentiate between hepatitis E and acute hepatitis caused by other infectious agents without the aid of laboratory tests. The history and epidemiological details are of great importance in reaching a presumptive diagnosis (Kudesia and Wreghitt, 2009).

2.6.2 Clinical course

Hepatitis E has symptoms of a self-limiting, acute icteric disease, similar to those caused by hepatitis A. Development of chronic hepatitis has not been observed. High mortality rate in pregnant women is often associated with massive hepatic necrosis (Kudesia and Wreghitt, 2009).

2.6.3 Complications

Fulminant disease, especially in pregnant women, is described in several epidemics, with 10–20% lethality (Kudesia and Wreghitt, 2009).

2.7 Immune response to hepatitis E

The immune response to hepatitis E appears late in the incubation period or during the acute phase of illness. It starts from the brisk rise of anti-HEV IgM and is followed by anti-HEV IgG. Anti-HEV IgM declines rapidly during early convalescence while anti-HEV IgG has been shown to persist for a long period of time (> 14 years) (Khuroo *et al .,* 1993).

2.8 Vaccination

Should one be infected by hepatitis E and recover, he or she will get protective immunity, with the courtesy of CD4 and CD8 T cells. Another way to induce immunity is via vaccination (Wedemeyer *et al*., 2012). The HEV vaccine which is in the most advanced stages of development is HEV 239. It is a Chinese manufactured vaccine that has a 94–100% efficacy in a phase III trial conducted onmore than 100,000Chinese soldiers.Although it is based on the type 1 genotype, it works even against genotypes 1 and 4. Response to genotype 3 is not known. It is still in the development stage to be used worldwide but has been approved for use in China (Zhu *et al.,* 2010).

2.9 Prevention and control

As almost all HEV infections are spread by the fecal-oral route, good personal hygiene, high quality standards for public water supplies and proper disposal of sanitary waste have resulted in a low prevalence of HEV infections in many well developed societies . For travelers to high endemic areas, the usual elementary food hygiene precautions are recommended. These include avoiding drinking water and/or ice of unknown purity and eating uncooked shellfish, uncooked fruits or vegetables that are not peeled or prepared by the traveler (Previsani and Lavanchy, 2001).
Strict hand washing should be adhered to, and where possible the patient should be put in a single room (Kudesia and Wreghitt, 2009).

2.10 Treatment

Acute hepatitis E in immunocompetent persons usually only requires symptomatic treatment, as almost all of them are able to clear the virus spontaneously. A report showed significant improvement of liver enzymes and functions in a patient with severe acute hepatitis E who was treated with ribavirin for 21 days. Although ribavirin therapy is contraindicated in pregnancy owing to teratogenicity, the risks of untreated HEV to the mother and fetus are high, and trials of antiviral therapy might be worthwhile (Kamar *et al*., 2012)**.**
In transplant recipients with chronic HEV infection, viral clearance is desirable. The first step is to reduce the immunosuppressive therapy, as reduction of immune-suppression results in viral clearance in 30% of patients. Antiviral therapy should be

considered for patients for whom immunosuppressive therapy cannot be reduced and for those who do not achieve viral clearance after reducing immune-suppression. Although data are limited, ribavirin mono-therapy (600–1000 mg/day) for at least 3 months seems to be the first treatment option for patients with chronic hepatitis E who are not able to clear HEV after immune-suppression is reduced.

Treatment with pegylated interferon alpha for 3-12 months has led to sustained clearance of HEV RNA in patients with chronic hepatitis E who underwent liver transplantations. However, interferon therapy can cause significant adverse effects and organ rejection in transplant recipients, especially those who have undergone heart or kidney transplantation (Kamar *et al*., 2012)**.**

2.11 Diagnosis of hepatitis E virus

Since cases of hepatitis E are not clinically distinguishable from other types of acute viral hepatitis, diagnosis is made by biochemical assessment of liver function (laboratory evaluation of: urine bilirubin and urobilinogen, total and direct serum bilirubin, ALT and AST, alkaline phosphatase, prothrombin time, total protein, albumin , IgG, IgA, IgM, complete blood count). Acute hepatitis E is diagnosed when the presence of IgM anti-HEV is detected (Previsani and Lavanchy, 2001).

The diagnosis of HEV infection is based on detection of HEV IgG and IgM antibodies in blood and of HEV RNA in blood and stool. Both HEV IgM and IgG ELISA based assays are available, but have not been standardised so far. Sensitivity and specificity may vary considerably between different assays and even between batches of a given assay. HEV IgM antibodies are detectable as soon as symptoms occur. IgG antibodies may reach a sensitivity of 72% to 98% and a specificity of 78% to 96% to diagnose HEV infection in immunocompetent patients,37 but are less accurate in immunocompromised individuals. HEV IgG may persist for years (DeNiet *et al*., 2012).

Storage of serum samples is acceptable for several days at 4°C, although anti-HEV will be preserved at -20°C, and a temperature of -70°C should be preferred when viremia is suspected. Hepatitis E should be suspected in outbreaks of waterborne hepatitis occurring in developing countries, especially if the disease is more severe in pregnant women, or if hepatitis A has been excluded. If laboratory tests are not available, epidemiologic evidence can help in establishing a diagnosis.HEV RNA can be detected in acute phase feces by PCR in approximately 50% of cases. Immune

electron microscopy is positive in only about 10% of cases. The viral proteins pORF2 and pORF3 have been expressed in various recombinant systems and form the basis for diagnostic tests and vaccine studies. To confirm the results of EIA or ELISA tests, Western blot assays to detect IgM and IgG anti-HEV in serum can be used, along with polymerase chain reaction (PCR) tests for the detection of HEV RNA in serum and stool, immunofluorescent antibody blocking assays to detect antibody to HEV antigen in serum and liver, and immune electron microscopy to visualize viral particles in feces (Previsani and Lavanchy, 2001).

Nucleic acid testing as a diagnostic marker of HEV infection has limitations in immunocompetent patients, because the period of viral shedding is limited to only one to two weeks around the time of ALAT elevation and jaundice.11 Still, HEV PCR is a crucial tool in immunocompromised patients . In these patients, the diagnosis can be easily missed as they often remain seronegative, whereas HEV RNA remains detectable during chronic infection. Furthermore,diagnostic testing for Hepatitis E in transplant patients may be delayed as increased levels of serum liver tests (ALAT,ASAT) are frequently seen as a consequence of drug toxicity or are related to other hepatotropic viral infections. Histological findings in liver biopsies may vary from portal hepatitis with dense lymphocytic infiltrates, piecemeal necrosis and fibrosis to cases with severe fibrosis or cirrhosis and are not specific for hepatitis E (DeNiet *et al*., 2012).

2.12 HEV and haemodialysis

Patients with chronic haemodialysis are always at risk of infectious diseases due to their compromised immune system , Moreover , these patients are at the frequent exposure to infectious agents during their visit to haemodialysis centers . Among viral infections the association of HEV with the parenteral transmission of the virus remains highly controversial (Omid *et al*., 2013).

2.13 Hepatitis E among haemodialysis patients in previous studies

Different studies carried out for Hepatitis E virus among haemodialysis;

In Iran 2010 , in cross sectional study they compare the seroprevalence of HEV among 80 patients with maintenance haemodialysis and 276 healthy individuals from Jahrom and shiraz , southwest of Iran serum samples were tested for the presence of

IgG antibody by enzyme immunoassay (ELISA) test , the seropositivity among studied patients (6.2) and (2.9) in controls group (Omid *et al*.,2013).

In Athens , Greece 1996 , serum samples from 420 HD patients and 316 healthy volunteers were tested for IgG and IgM antibodies to HEV (anti-HEV). Anti-HEV testing was done by an enzyme immunoassay (EIA) based on recombinant proteins of HEV (Abbott Labs). All anti-HEV IgG positive sera were confirmed using synthetic peptides . They detected Anti-HEV IgG was confirmed in 27/420 (6.4%) of the HD patients and in 7/316 (2.2%) of the reference group (P=0.007). However, multiple logistic regression analysis showed that the prevalence of anti-HEV IgG was not significantly higher in HD patients compared with the reference group, after controlling for age and sex. No patient was found positive for anti- HEV IgM. There was a statistically significant association with age (p=0.024), and higher prevalence was observed in female (P value =0.04) (Psichogiou *et al*., 1996).

In Taiwan , during february 2005 , serum sample obtained from 400 Taiwanese on chronic haemodialysis (group 1) , 400 sex and age matched healthy subject (group 2) and hospital patients (group 3) were tested for the IgG anti-HEV. They found the prevelnce of anti-HEV among the haemodialysis patients and the healthy controls were 31% and 8.9% respectively .The difference (22%) was statistically significant (P value<0.01) In comparison , the anti HEV in hospital patients hospital patients was 16% (Lee *et al* .,2005).

In Sudan studies on patients with acute hepatitis during the floods of 1988 in Khartoum demonstrated that infection was mainly due to HEV (58%) with low incidence of HAV infection (5.45%). Amongst children with acute hepatitis in Khartoum State concluded that HEV was also the commonest cause of acute clinical hepatitis among the pediatric population with HEV infection at 59%, HAV at 33.3%, and HBV at 2.6% (Mudawi, 2008).

 There is large outbreak of hepatitis E among a displaced population in Darfur ,Sudan in 2004 (Guthmann *et al*., 2006).

Few studies have reported sporadic hepatitis E virus infections during non- outbreak periods in Africa . In that study , the prevalence of HEV in Sudan was investigated in 432 patients with acute hepatitis from 12 localities in North Kordofan , and from 152 patients involved in smaller outbreak of hepatitis in neighbouring Darfur . HEV infection was diagnosed in 147 (25%) patients 98 from Kordofan and 49 from Darfur.

The mortality was 10%. HEV RNA was detected by quantitative real time polymerase chain (RT-qPCR) in 38 (26%) patients 22 from Kordofan and 16 from Darfur (Elduma *et al*., 2016).

Recent study was conducted in Sudan, have revealed a high frequency of anti-HEV IgG among Sudanese pregnant women in Khartoum (Al-Tayeb *et al*., 2014).

3. Materials and methods

3.1 Study design

This was case control study.

3.2 Study duration

This study had been conducted during October 2016 to February 2017.

3.3 Study area

This study was conducted in East Nile Hospital - Khartoum , Sudan.

3.4 Study population and Sample size

This study included haemodialysis patient (n =45) and healthy individuals (n=45) neither of the patients nor the healthy controls had symptomatic active infection or suffer from immunological disorders or consumed immunosuppressive drugs.

3.5 Ethical consideration

Approval to conduct this study was obtained from Sudan University of Science and Technology and also permission letter to collect specimen (Appendix 1)

Informed consent obtained from each individual. Every subject informed about the procedure before sample was taken.

3.6 Data collection methods and tools

Data was collected by direct interviewing questionnaires (Appendix 2).

3.7 Expermental work

3.7.1 Specimen collection

Five ml of blood collected from each haemodialysis patients and normal population in plain container left to clot for 15 minutes then centrifuged at 3000 rpm for 5 min, stored in refrigerator at -20°C and analyzed at once using ELISA technique.

Blood samples were obtained from patients before hemodialysis sessions.

3.7.2 Detection of HEV IgG antibodies

ELISA (Enzyme Linked Immunosorbant Assay)Kit-(EUROIMMUN ,Germany) was used.

3.7.2.1 Assay principle (Appendix 3)

3.7.2.2 Procedure

The following steps were done according to the instructions of the manufacturer.

Specimens preparation and dilution

The specimens (cases and control) were treated at the same manner, both were diluted 1:101 in buffer (provided with EUROIMMUN ELISA Kits) and mixed well by using vortex.

Sample incubation

From each 3 calibrators, positive control, negative control and diluted patient samples 100 µl were added into the individual microplate wells according to pipetting protocol.

The reagent wells were covered with protective foil and incubated at 37℃ for 60 minutes.

Washing

After the incubation, reagent wells were washed 3 times with 450µl of wash buffer (30-60 seconds per washing cycle) by using of TECAN Columbus washer.

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

Conjugate incubation

From enzyme conjugate (peroxidase-labelled anti-human IgG), 100µl was added into each of the microplate wells. The reagent wells were covered with protective foil and incubated for 30 minutes at room temperature.

Washing

After the incubation, reagent wells were washed 3 times with 450µl of wash buffer (30-60 seconds per washing cycle) by using of TECAN Columbus washer.

At the end of washing cycle, the strip plate was turned onto clean towel, and tapped to remove any remainders.

Substrate incubation

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

Stopping of the reaction

Hundred micoliter of stop solution (0.5 M Sulphuric acid) was added into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement

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

Calculation of the results

Results were evaluated semi quantitatively by calculating a ratio of the extinction value of the patient sample over the extinction value of calibrator 3 according to following formula:

Extinction of the control or patient sample

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 3}} = \text{Ratio}$$

Interpretation of the results

Ratio <0.8: negative

Ratio ≥0.8 to <1.1: borderline

Ratio ≥1.1: positive

3.8 Data analysis

All collected data were analyzed using SPSS(Statistical Package of Social Science) program(software version 16) , Pearson Chi-square test was used to analyze the data . P value <0.05 was considered statistically significant.

4. Results

A total of 90 participants 45 (50%) haemodialysis patients as cases and 45(50%) healthy individuals as control group were enrolled in this study . Their age ranged from 18-68 years , most of them 15(33.3%) were in age group 59 - 68 and 38 - 47 years among case and control group respectively.

Most of study population were males 35(77.8%) and 38(84.4%) among case and control group respectively , and most of them (86.7%) had haemodialysis for 2 years .

The overall frequency of HEV IgG antibodies among study population were 26 (57.8 %) and 16 (35.6 %) among case and control group respectively (Table 1) , with insignificant difference between haemodialysis (case) and healthy individuals (control group)(P value > 0.05).

Regarding gender the highest seropositivity was observed among females haemodialysis patients and control group respectively 6(60%)and 3(42.9%) , there was no significant association between HEV and gender (*P* value >0.05) (Table 2).

According to age group the highest seropositivity was observed among age group (58-67 years) , (38-47 years) among case and control group respectively with 8(30.8%)and 5(31.3%) frequency rate , with no significant difference between age groups and HEV (P > 0.05) (Table 3).

Table 1. Seoprealence of HEV among study population

HEV Study Population	Positive	Negative	Total
Case(Haemdialysis patients)	26(57.8%)	19(42.2%)	45(50%)
Control(Healthy individuals)	16(35.6%)	29(64.4%)	45(50%)
Total	42(46.3%)	48(53.3%)	90(100%)

Person Chi-square =4.464; P value = 0.057

Table 2 . Seroprevalence of HEV among study population according to their gender

HEV	Case		Total	Control		Total
	Males	Females		Males	Females	
Positive	20(57.1%)	6(60%)	26(57.8%)	13(34.2%)	3(42.9%)	16(35.6%)
Negative	15(42.9%)	4(40%)	19(42.2%)	25(65.8%)	4(57.1%)	29(64.4%)
Total	35(100%)	10(100%)	45 (100%)	38(100%)	7(100%)	45(100%)

Person Chi-square = .026; P value = 0.872 (P value >0.05).

Table (3) Seroprevalence of HEV among study population according to their age

Age group in years	Case		Total	Control		Total
	Positive	Negative		Positive	Negative	
18-27	3(11.5%)	1(5.3%)	4(8.9%)	2(12.5%)	8(27.6%)	10(22.2%)
28-37	5(19.2%)	5(26.3%)	10(22.2%)	4(25%)	7(24.1%)	11(24.4%)
38-47	6(23.1%)	3(15.8%)	9(20.0%)	5(31.3%)	10(34.5%)	15(33.3%)
48-57	4(15.4%)	3(15.8%)	7(15.6%)	4(25%)	3(10.3%)	7(15.6%)
58-67	8(30.8%)	7(36.8%)	15(33.3%)	1(6.2%)	1(3.5%)	2(4.4%)
Total	26(100%)	19(100%)	45(100%)	16(100%)	29(100%)	45(100%)

Person Chi-square = 1.148 ; P value = 0.887 (P value >0.05).

5.1 Discussion

Patients with chronic haemodialysis are always at risk of infectious diseases due to their compromised immune system . Moreover , these patients are at the frequent exposure to infectious agents during their visit to haemodialysis centers . Among viral infections the association of HEV with the parenteral transmission of the virus remains highly controversial (Omid *et al* ., 2013).

The present study investigated the prevalence of anti-HEV seropositivity in a selected population of chronic haemodialysis patients in East Nilel Hospital and compared finding with healthy individuals.

 The result revealed that seropositivity was more in haemodialysis   patient 26 (57.8%), it was different from control group16 (35.6%).

 This result is higher than of (6.2%) and (2.9%) in the study from southwest of Iran (Omid *et al.* ,2013) , 27 (6.4%) and 7(2.2%) in Athens, (Psichogiou *et al* ., 1996), 31% and 8.9% in Taiwan % (Lee. ,*et al* 2005) in studied patients and controls group respectively.

There was no association between HEV infection and haemodialysis in this study (P value > 0.05) , however it was different from result reported by (Lee. ,*et al* 2005) in which (P value <0.01), and similar to that reported by (Omid *et al*., 2013, and Psichogiou *et al* . , 1996) in which there is no association as (P value > 0.05).

The presence of anti HEV was associated with age and gender (P value <0.05)and higher prevalence was observed in females reported by (Psichogiou *et al* . , 1996) , in contrast there was no association between HEV infection with age and gender in the presence study (P value > 0.05).

Therefore , high variation in the results have delayed a comprehensive conclusion for the existence of pareneral HEV infection . This conflict should be resolved with more standardized global studies on different groups of people . Yet some findings could explain (at least in part) some of these disagreements . As mentioned , patients with chronic haemodialysis and renal disease suffer from immunocompromised condition such condition may cause weakening the immune response against infections , like HEV , and may cause the production of varied amounts of antibodies in the affected

patients . In addition to that , studies have shown a wide range of the persistence of anti HEV IgG antibodies in the circulation. Moreover; genetic variation of the virus can cause massive diversion of the immune responses in the infected patients .Only one single serotype has been reported for the HEV samples isolated from patients with hepatitis , however , there has been considerable genetic diversity among the isolates enough to subgroup the virus into four genotypes with different features and invasiveness . Genetic variations in HEV can cause more antigenic diversification in the isolates from different geographical locations . Effect of genetic variation could be shown by cellular immune response , moreover some of anti HEV antibody responses detected in patients or healthy individuals could be resulted from other infections , cross reacting with HEV antigens in serological tests as false positive results . more reasons can contribute for the variation in the results of serological tests of serological tests of anti-HEV antibody which needs to be found by further investigation (Omid *et al.* , 2013).

This high seropositivity may also be the result of a local HEV infection traveling is generally uncommon in chronic HD patients, also HD patients received packed transfusion (red blood cells) if their haemoglobin was low.

5.2. **Conclusion :**

This study suggests that chronic haemodialysis is not associated with an increased risk of exposure to HEV, and there is no association between HEV with age and gender .

5.3. **Recommendations:**

1. Positive results need to be confirmed with PCR.
2. The results emphasize the need to initiate more studies on the prevalence of HEV among HD patients in other parts of Sudan.
3. Education of haemodialysis patients to raise their awareness of attention to good hygiene to reduce the chances of infection.
4. A careful surveillance in the general population is required and further appropriate investigations are needed to identify the exact mode of transmission and risk groups in Sudan.

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Appendix (1)
Permission letter for specimen collection

Appendix 2

Questionnaire

Sudan University of Science and Technology

College of Graduate studies

Title: Seroprevalence of Hepatitis E Virus among Sudanese Haemodialysis patients attending East Nile Hospital – Khartoum

Prepared by : Samar Abd Elgani Mohamed Kheer

Supervisor : Dr Wafa Ibrahim Elhag

- Name …………………..   •Serial number…………………..
- Gender :
 Male ◯   Female ◯
- Age :
18-27 ◯
28-37 ◯
38-47 ◯
48-57 ◯
58-67 ◯

- *Duration of haemodialysis (years)...*

- Specimen:
Serum ◯   Other ◯

- Method: ELISA IgG for HEV
- Laboratory Findings:
Positive ◯   Negative ◯

Appendix (3)

Principle

The ELISA test Kit contains microtiter strips each with 8 break-off reagent wells coated with recombinant antigens of hepatitis E virus . in the first reaction step , diluted patient samples are incubated in the wells . in the case of positive samples , specific IgG antibodies (also IgA and IgM)will bind to the antigen . to detect the bound antibodies , a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalyzing a color reaction.

Appendix(4)

HEV IgG Microplate result
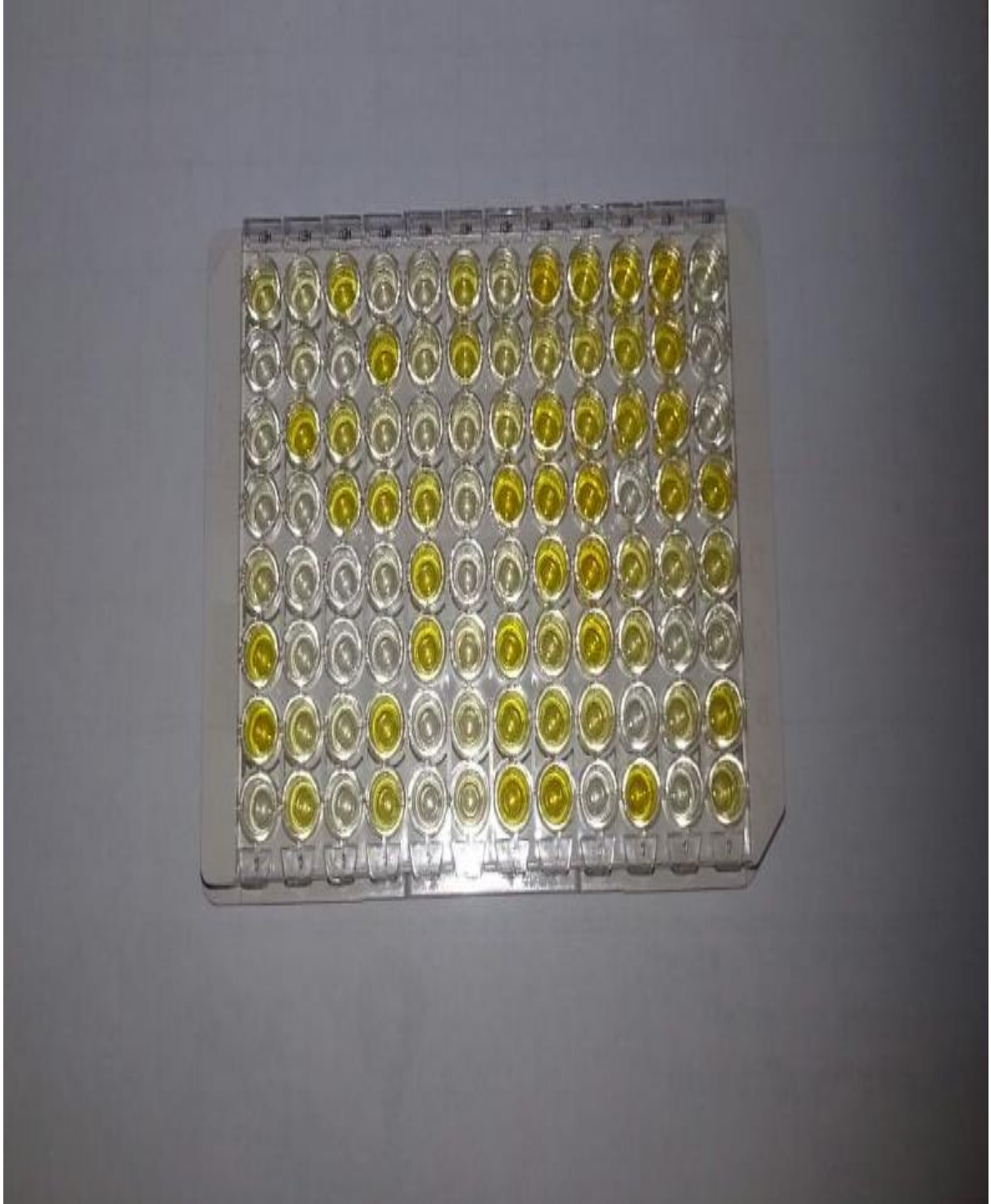
Appendix(5)

Anti-HEV ELISA(IgG) sheet