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Seroprevalence of HEV infection and risk factors among Sudanese pregnant women in Khartoum state

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Abstract

Background: *Hepatitis E virus* (HEV), a single-stranded, positive-sense RNA virus is responsible for acute Hepatitis E epidemics in many developing countries. HEV infection in pregnant women is more common and fatal in the third trimester. The incidence of acute viral *hepatitis E* is known for being the cause of major outbreaks of waterborne hepatitis in Africa. Traditional sanitation and water supplying systems are one of the most important factors for the virus transmission**.**

Objectives: our aims were to confirm the incidence of *Hepatitis E* virus among pregnant women attending Khartoum teaching hospitals and to detect the association of abortion in women with HEV infection, the relative risk factors and clinical symptoms.

Methods: Ninety-three pregnant women were enrolled in this study. Enzyme linked immunoassay (ELISA) was performed to determine the presence of anti-HEV IgG during the period from March to May 2015.

The results: HEV IgG antibodies were detected in 61.2% (57/93) of the women under study. The highest percentages were recorded in third trimesters of pregnancies 62.5% (34/57). HEV is associated with high rates of spontaneous abortion, we found about 36.8% (21/57) of HEV positive patients abortive with odd ratio above 1 which means that HEV is considered as risk factor for abortion among pregnant women. While other risk factors include water system supplies, age groups (26-45), rural residence, history of blood transfusion and travelling out of Sudan were 71.9% (41/54), 54.4% (31/57), 50.9% (29/57), 12.3% (7/57) and 5.3% (3/57) respectively. The symptoms were variable with no significant interpretations.

Conclusion: In this study, we found a high frequency of anti-HEV IgG among Sudanese pregnant women in Khartoum which is endemic in Sudanese habitant original root that had never travelled out of the country. Also the present study indicated that one of the highest risk factor is water hygiene cautions. HEV positive patient's symptoms are not reliable for HEV diagnosis which needs more sensitive and specific diagnostic tools to confirm the result.

Keywords: Anti-HEV IgG, ELISA, *Hepatitis E virus*, pregnant women, Sudan.

Introduction

Described as an inflammatory liver disease agent, *Hepatitis E virus* (HEV) is a spherical, non-enveloped, and single-stranded RNA [1, 2]. *Hepevirus* is genus of *Hepatitis E virus* [3]. *Hepatitis E virus* causes endemic, but also large epidemic infection. Four modes of transmission of HEV infection have been reported: fecal-oral transmission, food-borne transmission, blood-borne and vertical transmission [4, 5]. The most common mode of transmission of HEV, also responsible for the majority of the HEV infection out breaks, is through the fecal-oral route, usually by ingestion of contaminated water. Potential exists for food-borne transmission and some cases have been observed where consumption of raw or uncooked meat from wild boar and deer has led to HEV infection. Blood-borne transmission is rare but has been documented in some cases involving blood transfusion. Some cases of vertical (parental) transmission from mother-to-child have been documented, particularly in India, but this is considered to be of minor importance as a mode of transmission for HEV and more investigation is required. Person-to-person transmission and secondary household cases are uncommon, particularly in epidemic (poor hygienic) conditions. In non-endemic regions, where

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autochthonous cases have been observed, zoonotic transmission has been considered as the likely mode of transmission, but more investigation is required [5]. HEV is a major cause of hepatitis transmitted by the fecal oral route. It is a common cause of waterborne epidemics of hepatitis in Asia, Africa, India and Mexico but not in the United States [6]. HEV is first documented in samples collected during the Delhi outbreak of 1955, when 29,000 cases of icteric hepatitis occurred after sewage contaminations of the city, drinking water supply [7]. Outbreaks of disease attributable to drinking water are not common in the U.S., but they do still occur and can lead to serious acute, chronic, or sometimes fatal health consequences, particularly in developing countries [8, 9]. Sudan has been reported as one of the developing country with unprotected water and poor sanitation, hygiene have been reported to be ranked third among the 20 leading risk factors for health burden. In addition to that, Sudan is a large country with rich sources of water supply including Hafir, tank, zeer. Lake and stream are the main sources of drinking water in Sudan, especially in the rural areas, and not only used for human. Consumption is also used for animals and accordingly this is the common source of contamination with harmful microorganisms [10]. The pathogen in study is in charge of at least 50% of acute non-Anon-B hepatitis in developing countries and major cause of human viral disease with clinical and pathological features of acute hepatitis [10, 11]. In many countries in Africa, including South Sudan and in Asia *Hepatitis E virus* has been reported as the major cause of clinical hepatitis. Four human genotypes of mammalian HEV have been reported and are recognized; genotypes 1 and 2 are restricted to human infection, whereas genotypes 3 and 4 are zoonotic. Pigs are a known reservoir for HEV, and several other animal species, including deer, rabbits and mongooses may potentially serve as HEV reservoir [12]. *Hepatitis E* infection during pregnancy and in the third trimester, especially with genotype 1, is associated with more severe infection and might lead to fulminant hepatic failure and maternal death [13, 14]. Although the mechanism of liver injury is not yet clear, it is possible that the interplay of hormonal and immunologic changes during pregnancy, along with a high viral load of HEV, render the woman more vulnerable [15]. Immunologic changes during pregnancy promote the maintenance of the fetus in the maternal environment by suppression of T cell-mediated immunity, rendering pregnant women more susceptible to viral infections like HEV infection. During pregnancy, levels of progesterone, estrogen and human chorionic gonadotropin increase as pregnancy advances. These hormones play a considerable role in altering immune regulation and increasing viral replications [16]. *Hepatitis E* infection with genotype 1 during the third trimester can lead to maternal mortality in 15% to 25% of cases [17]. Most of the studies showing high maternal mortality are from India, where infection occurs in epidemics. There is a very high risk of vertical transmission of HEV from mother to fetus. During a Delhi epidemic, a hospital based study revealed that HEV infection during pregnancy was associated with miscarriage, stillbirth, or neonatal death in 56% of infants [18]. One recent study highlights that HEV infection might be responsible for 2400 to 3000 stillbirths each year in developing countries, with many additional fetal deaths linked to antenatal maternal deaths. There is a very high risk of preterm delivery in pregnant women with HEV infection, with poor neonatal survival rates [13, 19]. In two separate studies from India, 15% to 50% of live-born infants of mothers with HEV infection died within 1 week of birth [11, 20]. During an outbreak in Sudan from 2010 to 2011, among 39 pregnant women with HEV infection there were 14 intrauterine deaths and 9 premature deliveries [21]. In the internally displaced population camps of Darfur-Western-Sudan a large outbreak of hepatitis E virus infection was reported in June 2004 [22]. It affects primarily young adults and is generally mild [19, 23]; however the mortality rate is higher among pregnant women, especially during the second and third trimesters of pregnancy [24, 25]. In Sudan an incidence fatality ratio of 17.8% was declared in an outbreak in Darfur during 2004, with a ratio of 31.1% among pregnant women [26]. HEV infection can be examined using different methods firstly by detection of viral particles in stool. Secondly by using electron microscopy or sero-diagnosis of anti-HEV antibodies in serum like *Hepatitis A virus* [27].

The aims of this study were to verify the frequency of *Hepatitis E virus* among pregnant women attending Khartoum hospitals, to detect association of abortion with HEV and to evaluate the major risk factors and clinical symptoms relevant with such kind of viral disease.

Methods

This is a cross-sectional study conducted in Khartoum teaching Hospital, Sudan during the period of March-May 2015. Ninety-three pregnant women, who attended Khartoum teaching Hospital in Sudan, were recruited in this study. This study was approved by Omdurman Islamic University ethical committee board and an informed consent was obtained from each patient before collecting the demographic and clinical data via questionnaires including age, place of residence, stage of trimester during pregnancy, travelling history, drinking water sources and symptoms which include history of suffering liver diseases, jaundice, vomiting, abdominal pain, hepatomegaly, diarrhea headache, fever, dark urine. Five ml blood samples were obtained by vein-puncture for serological analyses. Samples were centrifuged and sera were separated immediately and stored at -20˚C then tested for the presence of anti-HEV IgG antibody by enzyme-linked immuno-sorbent assay (ELISA) kits.

The results were scored as positive or negative according to the standard procedures recommended by the manufacturer (DS-EIAANTI-HEV-G) Diagnostics System (Soronno, Italy). Positive and negative controls were included in all the ELISA microplates assayed. The cutoff was calculated by adding 0.20 absorbance units to the negative control value, or mean value. The results are considered positive when clinical sample absorbance value equal or more than the cut-off value 0.200. Internal quality controls were concerned using also standard positive and negative sample.

Statistics

All collected data were analyzed using SPSS. To evaluate the relationship between different factors we performed chi-square analysis. P value <0.05 were considered statistically significant. Odd ratio was calculated.

Result:

During the study period between 21st March 2015 and 22nd May 2015 a total of 93 pregnant women who attended Khartoum teaching hospital, Sudan, were enrolled in the current study. All the patients were found to be healthy on routine antenatal medical examination. Overall HEV IgG sero-prevalence rate among pregnant women in Khartoum, Sudan over the two month period was 61.2 % (57/ 93) (Table 1; Figure 1-a).

Table1. Baseline characteristics of pregnant women enrolled in the study (N=93)

Characteristic	NO	Percentage (%)
Study group	**93**	**100**
Distribution of Anti-HEV IgG antibodies among pregnant women		
Positive	**57**	**61.2 %**
Negative	**36**	**38.7 %**

The age distribution of pregnant women sero-positive for HEV IgG ranged from 15 to 45 years and the median age was 37 years. The sero-prevalence was high 54.4% (31/57) among pregnant women of 26-45 years age, followed by 45.6% (26/57) 15–25 years group (Figure1-b). However, there was no statistically significant correlation between increasing age and HEV sero-positivity and no statistically significant difference in prevalence by age group. The seroprevalence was also higher in pregnant women in the third trimester (54.4% (34/57) than in the second and first trimester. About (36.8% (21/57) of HEV positive pregnant had history of abortion with odd ratio above 1 and high percentage of water system supplies, age groups (26-45), rural residence, history of blood transfusion and history of travelling out of Sudan were 71.9% (41/54), 54.4% (31/57), 50.9% (29/57), 12.3% (7/57) and 5.3% (3/57) respectively. (Figures: 1-a,1-b,1-c,1-d,1-e,1-f,1-g).

Despite the high HEV prevalence among pregnant women, symptoms and signs compatible with acute viral hepatitis were not found frequently connected with history of suffering liver diseases 0% (0/57), jaundice 8.8% (5/57)**,** vomiting 35.1% (26/57), fever 36.8% (21/57), abdominal pain 42.1% (24/57), dark urine 7% (4/57), diarrhea 21.1% (12/57), and headache 52.6% (30/57) (Figures: 2-a, 2-b, 2-c, 2-d, 2-e, 2-f, 2-g,2-h).

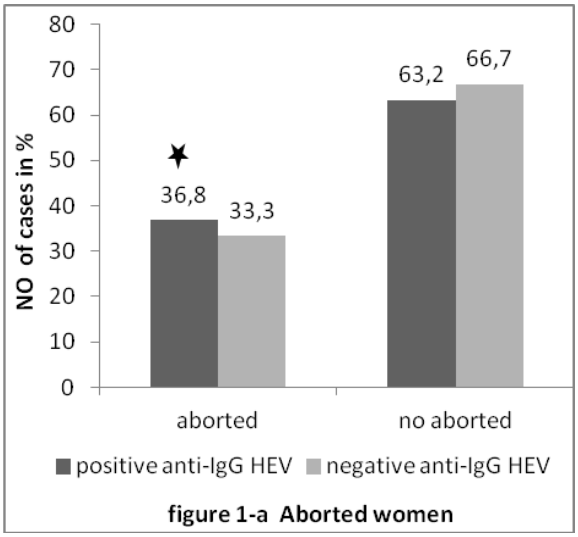

figure 1-a Aborted women

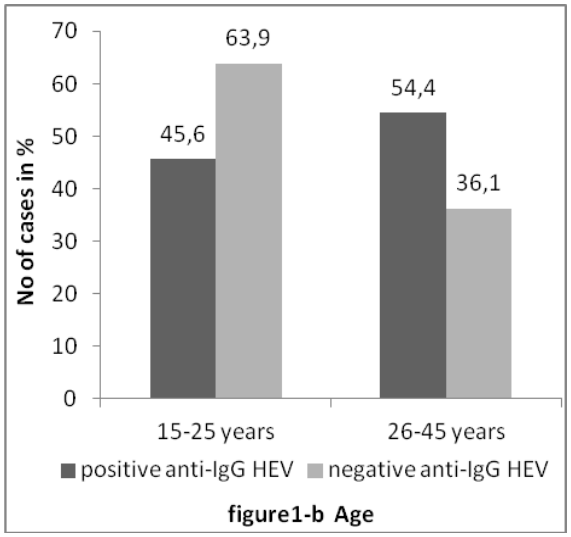

figure1-b Age

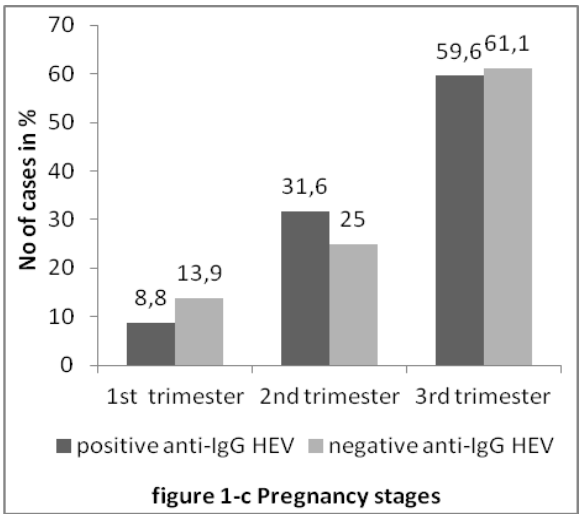

figure 1-c Pregnancy stages

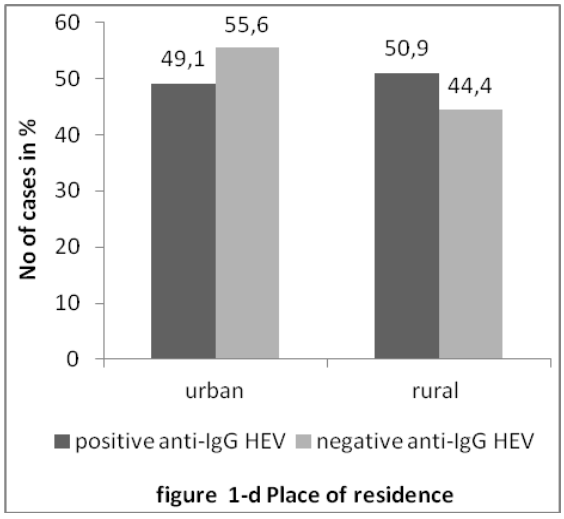

figure 1-d Place of residence

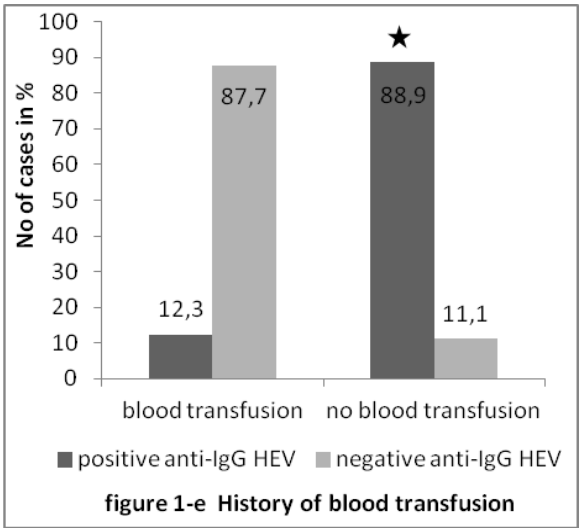

figure 1-e History of blood transfusion

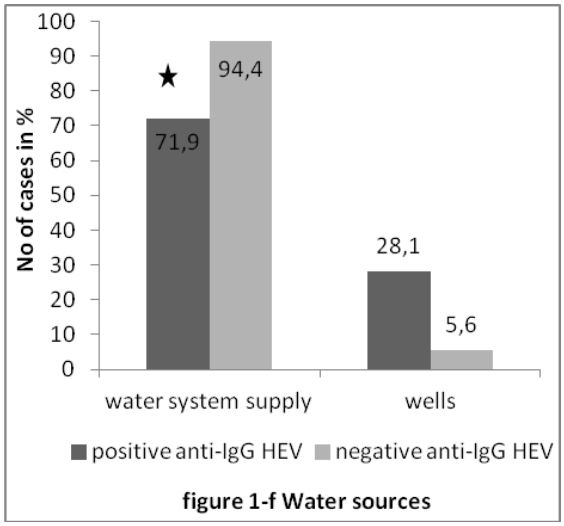

figure 1-f Water sources

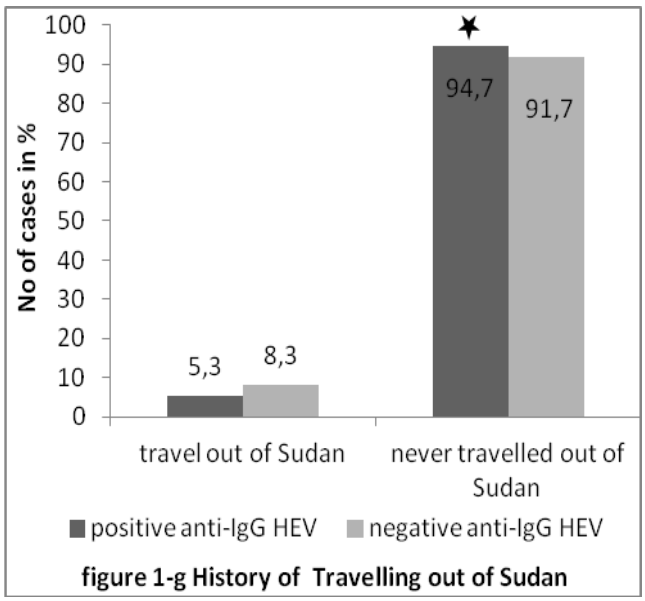

figure 1-g History of Travelling out of Sudan

Figure 1. Percentage of total number (57) HEV positive pregnant women from total samples (93) in relation to many predisposing factors.

Factors included; abortion, ages, duration of pregnancy, residence; urban, rural, history of blood transfusion, source of drinking water; water supplementary systems, wells and travelling out of Sudan. Chi-square analysis was used and revealed

statistically significance with p value < 0.05 labeled with symbol (*)
confined for abortion, history of blood transfusion, source of water and travelling out of Sudan as shown in the figure 1-a ,1-e, 1-f and 1-g above and odd ratio above 1. While the other factors exhibited statistically no significance (ns) with p value > 0.05 figure, 1-b, 1-c and 1-d.

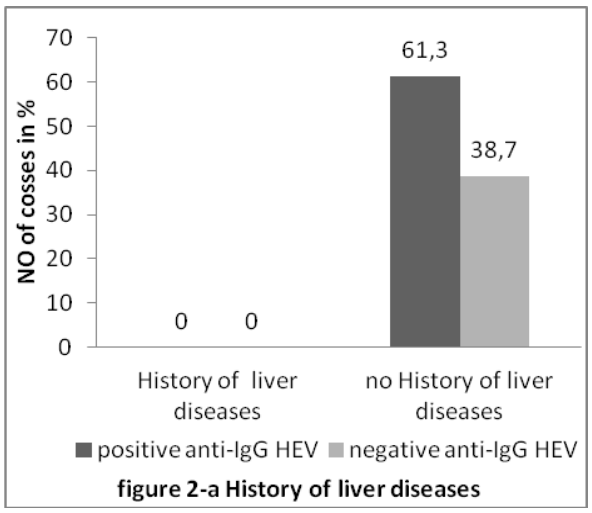

figure 2-a History of liver diseases

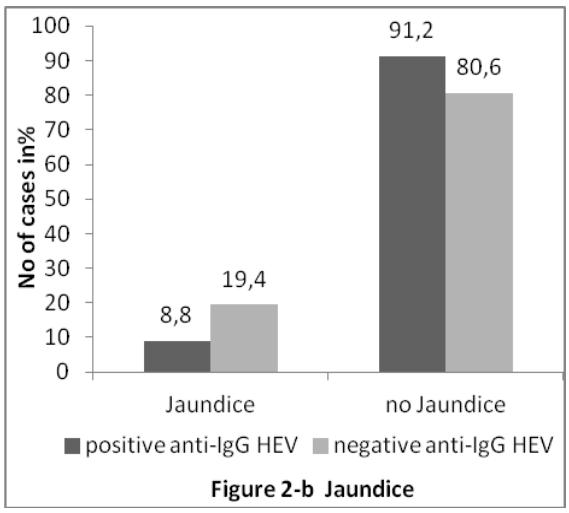

Figure 2-b Jaundice

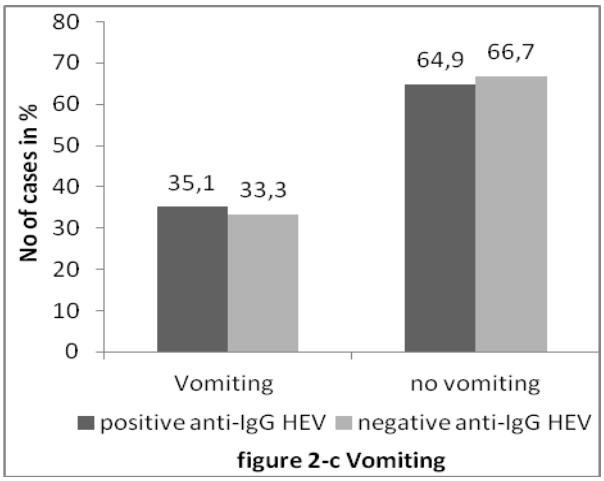

figure 2-c Vomiting

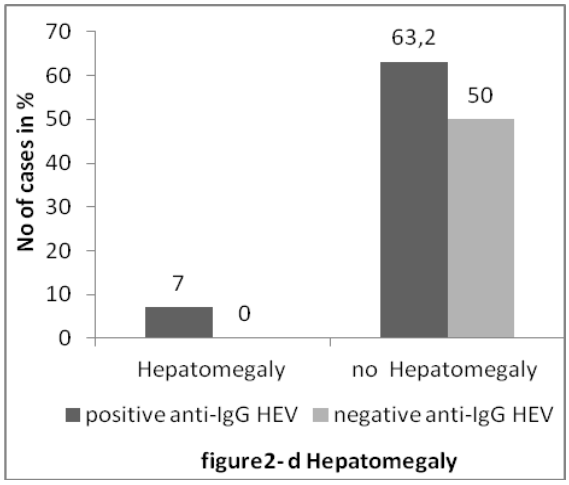

figure2- d Hepatomegaly

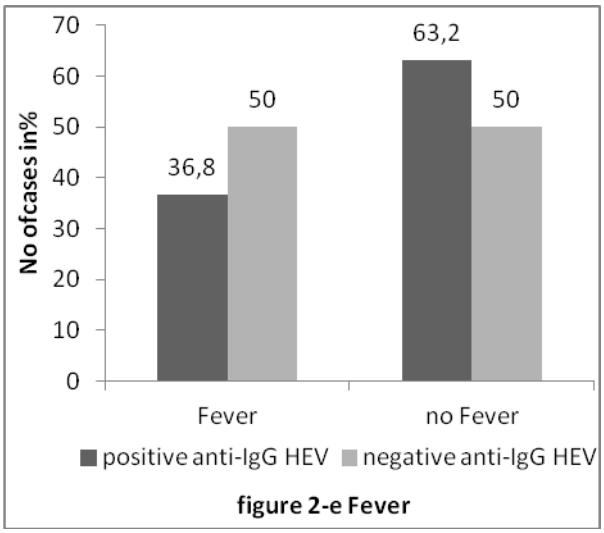

figure 2-e Fever

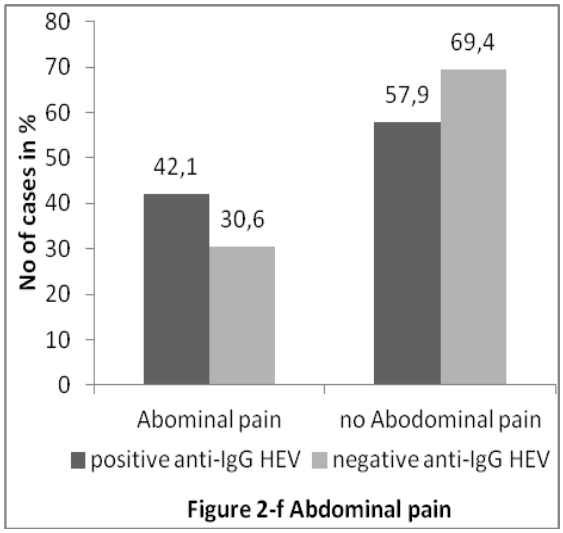

Figure 2-f Abdominal pain

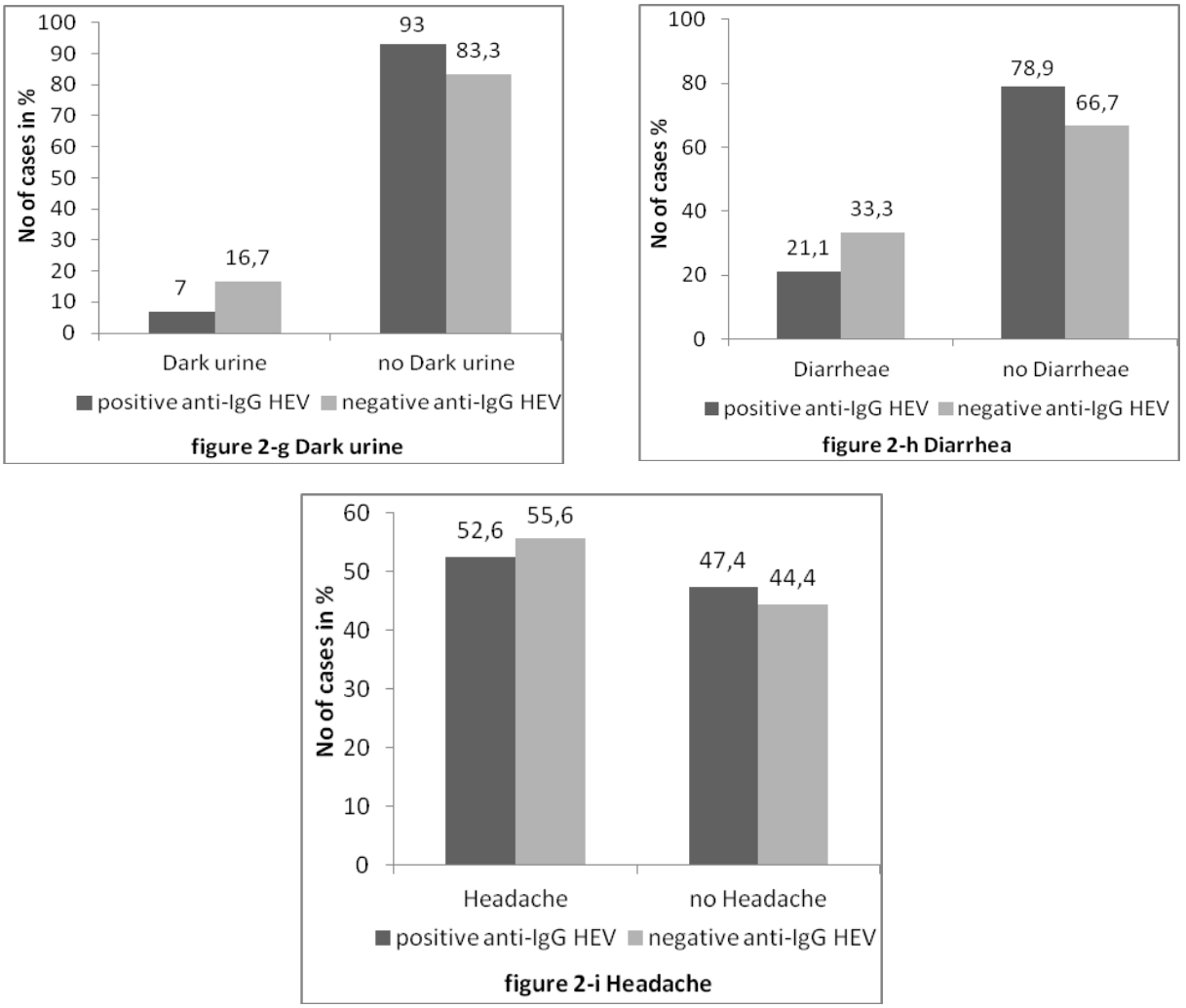

Figure 2. The percentage of HEV positive pregnant women associated with clinical manifestation symptoms. The symptoms studied include; Asymptomatic history of liver disease, jaundice, vomiting, hepatomegaly, fever, abdominal pain, dark urine, diarrhea and headache. Chi-square analysis was used and revealed statistically no significant with p value > 0.05 as shown in the subfigure 2-a, 2-b, 2-c, 2-d, 2-e, 2-f, 2-g, 2-h and 2-i and odd ratio less than 1

Discussion

This study found very high overall frequency rates (61.2% (57/93) of HEV antibody among pregnant women, suggesting the possibility of subclinical infections. The overall frequency of HEV among pregnant women attending Khartoum teaching hospital is higher than that found in Darfur, Western-Sudan (31.1%), Khartoum hospitals (41.1) and India (60%) and lower thanthat in Egypt (84.3%) [28, 29, 30]. Hepatitis E in pregnancy is also associated with high rates of spontaneous abortion, intrauterine death, and preterm labour [31].

Hepatitis E virus is associated with abortion during pregnancy, we found about (36.8% (21/57)) of HEV positive patient was abortive. The high seroprevalence of HEV in pregnant women at Khartoum teaching hospital may suggest wide spread among pregnant women in this country and therefore it is reasonable to speculate that HEV may circulate in general population and this calls for population based study to confirm this speculation. Furthermore, the high positive rates of seroprevalence of anti-HEV IgG among pregnant women in third trimesters were 59.6% (34/57) and this agreed with many different previous studies [29, 30, 31].

In addition to that, in the present work, the seroprevalence of anti-HEV IgG among pregnant females is higher with water supply than wells sources (71.9% (41/57) vs 28.1% (16/57)) respectively which imply that infected pregnant women by HEV are slightly more in rural area than urban area (50.9% (29/57) vs. 49.1% (28/57)) respectively [32]. The virus is transmitted through the fecal-oral route of an infected person getting into the mouth, referred to as the fecal –oral route, may be direct from person to person by contaminated finger or indirect through food or water. The proportion of people with access to adequate water and continued sanitation has not increased due to population growth, insufficient continued investment, insufficient system, and lack of training

and spares to maintain clean working system. This result may be attributed also to high pressure of water inside the network which supplies Sudan area during the season. This pressure broke down and corrodes old metallic pipes and become a major source of contamination by feces. Also the incidence of acute viral *Hepatitis E* increases after floods as this allows sewerage contamination of piped and ground water as our study period coincides with rainy seasons and this agreed with previous study in Bangladesh and in southwestern Vietnam [33,34]. In addition to that low frequency in wells water usually contains few microorganisms because microbes are filtered out as the water trickles through the layers of the earth and also Temperature, pH and turbidity, together with the infiltration and percolation of surface water, which takes place in the wet season, seemed to be the driving factors in the shaping and selection of the microbial community and deterioration of water quality [35]. Until now, no epidemic cases of HEV infection have been reported in Khartoum, despite the high prevalence of IgG antibodies to this virus. Furthermore, no signs and symptoms compatible with acute viral hepatitis were found that would indicate past HEV infection. Probably, the initial HEV infection occurred early in life with early childhood exposure to hepatitis A virus in countries where it is somewhere endemic and reservoir in domestics. Our finding disagreed with previous study in Italy [36], Turkey [37] and Spain [38] where higher circulation of HEV was observed in immigrants or travelers abroad than native civilian.

Therefore, epidemiological studies of various age groups and in children are also needed. The interaction of Hepatitis E and pregnancy is fascinating and has provided new insights into the pathophysiology and understanding of the immunology and host susceptibility factors and their interaction to trigger the disease process. The severe liver injury due to HEV infection during pregnancy may be related to several possible factors, such as differences in immune and hormonal factors occurring during pregnancy, the genetic and environmental factors with its occurrence in certain developing countries [39]. The absence of screening for HEV antibodies in pregnant women, in blood and organ donors in most countries is based on the low risk of associated diseases like HIV. Recently HEV screening program has been recommended as part of the routine in several countries. The antenatal screening program also should be performed to minimize prenatal HEV transmission in order to take further precaution and protect fetus's life [40].

Conclusion and Recommendation

The high seroprevalence of HEV in pregnant women at Khartoum teaching hospital may suggest wide spread among pregnant women in this country, and Hepatitis E virus was associated with abortion during pregnancy. Asymptomatic history of liver disease, headache, abdominal pain, vomiting, diarrhea, jaundice, fever, hepatomegaly and dark urine in percentage of 61.3%, 52.6%, 42.1%, 36.8%, 35.1%, 21.1%, 8.8% and 7% respectively revealed no statistically significant association to HEV disease. Therefore, systematic application of hygiene measures is highly recommended to avoid exposure to this virus.

HEV infection is a major cause of morbidity in areas of overcrowding and poor water hygiene. Pregnant women and patients with preexisting liver disease are most likely to develop fulminant hepatitis, with an associated high mortality. Specific programs could therefore be targeted and strategies for HEV vaccination should be developed. HEV screening program has been recommended as part of the routine. Ultimately prevention of transmission by good sanitation and boiling drinking water is the best approach to reduce morbidity and mortality from HEV and a number of other waterborne pathogens. Care should be taken to prevent contamination of drinking water supply system from sewerage during construction of road and house.

Acknowledgements

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