Frequency of HEV antibodies in pregnant women and its association with existent risk factors, Sudan: A cross-sectional study

Abstract

Background: Hepatitis E virus (HEV) infection is an increasing global problem and high endemicity is observed in many developing countries. Pregnancy predisposes women to chronic infection and various complications **Objective:** This study aimed to investigate sero-positivity of HEV and existent risk factors among pregnant women in Gezira State, Sudan **Methods:** A cross-sectional design was followed, and a non–probability convenience sampling technique was used to collect 120 venous blood samples from pregnant women. Separated serum specimens was used for the quantitative detection of specific anti-HEV IgM and IgG by competitive ELISA method (MIKROGEN GmbH - Germany) **Results:** The frequency of positive results for HEV IgG antibodies in the study population amounted to 44.2 (53/120). Of the 53 HEV IgG positive pregnant participants 47.2% (25/53) were in the third trimester period, 39.6% (21/53) had preterm labor, 39.6% (21/53) had a history of abortion and 24.5% (13/53) with jaundice. Significant association was found between HEV IgG positive women and preterm labor (p-value 0.004), and history of jaundice (p-value 0.009) **Conclusion:** This preliminary study highlighted the high frequency of sero-positivity of hepatitis E virus in pregnant women in the studied area of Sudan.

Key words: Hepatitis E, Pregnant women, Risk factors, ELISA, Sudan

Introduction

Hepatitis E virus (HEV) infection is an emerging, worldwide inflammatory liver disease [1]. The severity of HEV infection appears during pregnancy, which makes the disease a heavy burden, especially in developing countries with high endemicity [2]. Unlike other viral types of hepatitis, such as B and C, type E is transmitted through the fecal-oral route, which led to its identification for the first time by a Russian scientist [3]. There are 4 main genotypes of HEV; 1, 2, 3 and 4. HEV1 and HEV2 are responsible for poor sanitation transmission in developing countries [4], while genotypes 3 and 4 are usually zoonosis in developed countries such as western and central Europe [5].

The risk factors for the disease are related to the method of transmission, so it is related to food, drink, and the environment contaminated with the virus-carrying feces [6].

The groups most at risk of infection with the virus and the occurrence of complications are pregnant women and immunocompromised individuals. Pregnancy increases the multiplication of the virus by reducing immunity [7]. Sstudies have shown that the mortality rate of pregnant women due to HEV infection is higher than that of men and women who are not pregnant, especially in the third trimester [8]. In addition HEV infection during pregnancy can resulting in spontaneous abortion [9].

In the year 2004, an outbreak in Sudan was described as huge among the displaced in the western Darfur region, 2621 cases were recorded within six months, and deaths occurred, especially among pregnant women [10]. Recently, an outbreak of the HEV was documented among Ethiopian refugees in the Tigray border area between Sudan and Ethiopia [11], and such epidemics can move into Sudan because of the nature of the open borders and the ease of penetration into the interior.

Methods

Design and settings

This was a descriptive cross-sectional study aimed at investigating sero-positivity of HEV among pregnant women in Gezira State, Sudan from August 2016 to November 2018. Gezira State is inhabited by a mixture of races and tribes from inside and outside Sudan. Gezira became the Sudan's major agricultural region, with more than 2.5 million acres (10,000 km^2) under cultivation (Sudan.gov). This study included the following localities; Greater Wad Medani, Elkamleen, El Managil, South Gezira, Um-Alqura, Eastern Algezira, and Elhassahesa. Ministry of health ethically approved the study and informed consent was collected from each participant.

Sampling

The study was based on a non–probability convenience sampling technique during the attendance of pregnant women in Gezira State. A total of 120 venous blood specimens were obtained from pregnant women of different ages. Then Separated serum was analyzed for specific anti-HEV IgM and IgG using a competitive enzyme-linked immune-sorbent assay (ELISA) (MIKROGEN GmbH - Germany).

HEV IgG and IgM detection

According to the instructions of the manufacturer; sample absorbance was divided by the cut-off value of the assay, where, cut-off value = Nc + 0.50. Nc = the mean absorbance

value for three negative controls. The result of anti-IgM assay was considered negative when absorbance ratio/cut-off was <1, positive when absorbance ratio/cut-off is >1), and, equivocal when absorbance ratio/cut-off is 0.9 - 1.1. While for the anti-IgG assay the results were considered negative when absorbance ratio/cut-off was <1, positive when absorbance ratio/cut-off is >1 and equivocal when absorbance ratio/cut-off is 0.9 - 1.1. The equivocal results were retested to confirm the reactivity and the persistent results of the absorbance.

Ratio/cut-off of 0.9 - 1.1 was regarded as positive.

Statistical analysis

Collected socio-demographics and clinical data were analyzed by a computer system using statistical package for social sciences (SPSS-s) program using the Chi-square test and cross-tabulation. Statistical significance was set at *P*. values < 0.05.

Results:

In total, 120 pregnant women from seven localities were enrolled. Ages ranged from 17 to 45 years, with a mean of 28.23 years. The age group of less than 30 years represented 55.8% (67/120). The most frequent localities were Elhassahesa 40.8% (49/120) and Greater Wad Medani 32.5% (39/1200). Housewives accounted for 76.9% (92/120) of the participants. Primary and secondary education together observed among 63.3% (76/120). Wells as a water source was reported in 79.2% (95/120). HEV IgG positively detected in 44.2% (53/120), and a significant association was found only between positive HEV IgG and localities with p-value of 0.03. (Table 1).

Of the 53 HEV IgG positive pregnant participants 47.2% (25/53) were in the third trimester period, 39.6% (21/53) had preterm labor and 39.6% (21/53) had a history of abortion. 24.5% (13/53) of IgG positive participants suffered from jaundice. The majority of study subjects consumed raw meat, with a rate of 90.8% (109/120), and an IgG positivity of 88.7% (47/53). The significant association was documented between IgG positivity and preterm labor (p-value 0.004), and history of jaundice (p-value 0.009) (Table 2).

For IgM detection, a positive reaction was obtained only from 4 specimens with a percentage of 3.3% (4/120). Noted that, all IgM positive specimens gave positive result for IgG.

Table 1. Socio-demographics of pregnant women subject (No 120) and association with HEV IgG positivity

Socio-demographic		Frequency (%)	Positive IgG	p-value
Age	Less than 30 Years	67 (55.8)	25	0.089
	30 Years and more	53 (44.2)	28	
	Total	120 (100%)	53	
Locality	Elkamleen	8 (6.7)	5	0.03
	Elhassahesa	49 (40.8)	13	
	Eastern Algezira	5 (4.2)	3	
	Um Elqura & South Algezira	14 (11.7)	8	
	Greater Wad Medani	39 (32.5)	20	
	Elmanagil	5 (4.2)	4	
	Total	120 (100%)	53	
Residence	Urban	52 (43.3)	24	0.701
	Rural	68 (56.7)	29	
	Total	120 (100%)	53	
Occupation	House wife	92 (76.7)	38	0.507
	Worker	21 (17.5)	11	
	Employee	7 (5.8)	4	
	Total	120 (100%)	53	
Education level	Uneducated	11 (9.2)	2	0.127
	Primary	35 (29.2)	15	
	Secondary	41 (34.2)	23	
	University	33 (27.5)	13	
	Total	120 (100%)	53	
Water source	Wells	95 (79.2)	41	0.909
	River	23 (19.2)	11	
	Canals	2 (1.7)	1	

	Total	120 (100%)	53	
Hygiene	Good	85 (70.8)	41	0.162
	Bad	35 (29.2)	12	
	Total	120 (100%)	53	

Table 2. Clinical and risk factors of pregnant women subject (No 120) and association with HEV IgG positivity.

Risk factor		**Frequency (%)**	**Positive IgG**	**p-value**
Pregnancy period	First trimester	34 (28.3)	14	0.652
	Second trimester	35 (29.2)	14	
	Third trimester	51 (42.5)	25	
	Total	120 (100%)	53	
Preterm labor	Yes	32 (26.7)	21	0.004
	No	88 (73.3)	32	
	Total	120 (100%)	53	
Abortion	Yes	42 (35.0)	21	0.345
	No	78 (65.0)	32	
	Total	120 (100%)	53	
Jaundice	Yes	18 (15.0)	13	0.009
	No	102 (85.0)	40	
	Total	120 (100%)	53	
History of viral hepatitis	Yes	4 (3.3)	1	0.432
	No	116 (96.7)	52	
	Total	120 (100%)	53	
History of blood transfusion	Yes	21 (17.5)	11	0.404
	No	99 (82.5)	42	
	Total	120 (100%)	53	
Consumption of raw meat	Yes	109 (90.8)	47	0.467
	No	11 (9.2)	6	
	Total	120 (100%)	53	
Animal contact	Yes	78 (65.0)	33	0.576
	No	42 (35.0)	20	
	Total	120 (100%)	53	
Dietary intake	In side home	103 (85.8)	47	0.427
	Outside home	17 (14.2)	6	

	Total	120 (100%)	53	

Discussion

Many international documents predict the risk of infection with the hepatitis E virus, especially with the availability of means of transmission in developing countries [12], despite this, the information required to understand the disease in terms of sources, modes of transmission, risk factors and accurate diagnosis is almost non-existent in Sudan.

This study showed a high rate of IgG sero-positivity among the pregnant women subject, and the percentage reached 44.2%, which is an indicator of the viral spread in the study area, especially when compared to the rates of disease prevalence in the countries with the highest infection, which amounted to 30% in asymptomatic pregnancies and 49% in symptomatic pregnancies [2]. This HEV IgG sero-frequency is of important value especially as it reflects the infection prevalence status, consistent in China in Chongqing, a surveillance study concluded that there was a gradual increase over the period from 2012 to 2020 in IgG antibody [13]. Lower rates of IgG sero-positive were observed during pregnancy in central Nigeria [14], Ethiopia [15] and South Africa [16]. Also much lower results among adults and children were found in France [17] and USA [18].

In the present findings, IgM antibodies against HEV expressed a low frequency with a percentage of 3.3%. Such IgM-negative results excluded the possibility of acute HEV infection among the study subjects even in the presence of IgG positivity, while at the same time not excluding previous infection, however, a patient infected with hepatitis E virus during the window period or who is immunocompromised may also appear negative for IgM [19]

The results of the study found that there was a relationship between the positivity of HEV IgG antibodies and studied localities, and this necessitates the importance of conducting studies to find out the spread of the disease and the susceptibility of the population to

identify the most infected areas and the underlying risk factors. In line, HEV IgG was associated with rural residence and animal contact in France among blood donors [20]. In rural China, a study suggested that infection with genotype 4 is most likely to occur through contact with pigs [21]. No significant association was observed between age group of pregnant women in the study and IgG detection, similar study pointed at this [22], but different from those recorded in India by [23].

There are many indications that infection with the HEV during pregnancy results in poor outcomes [24] [2]. Among pregnant women, infection by the HEV was more common in the third trimester of gestation [24]. Statistical analysis of the present results showed that there is a relationship between positive HEV IgG antibody and jaundice or preterm birth, similar statistics through the world documented these cases [25] [26] [14]. Taking into account such data, additional precautions can be taken to monitor pregnant women, such as screening for the HEV.

Conclusion

This is one of the few studies that has shown the sero-frequency of HEV in a sample of pregnant women in Sudan.

Study limitation

This study did not identify common HEV strains and their association with risk factors**.** Also, the sample size does not represent the population of the study State, and therefore the study did not determine the prevalence rate.

References

1. Chaudhry SA, Verma N, Koren G. Hepatitis E infection during pregnancy. Can Fam Physician. 2015 Jul;61(7):607-8. PMID: 26175368; PMCID: PMC4501603) (Wu, C., Wu, X. & Xia, J. Hepatitis E virus infection during pregnancy. *Virol J* **17**, 73 (2020). https://doi.org/10.1186/s12985-020-01343-9.
2. Bigna, J.J., Modiyinji, A.F., Nansseu, J.R. *et al.* Burden of hepatitis E virus infection in pregnancy and maternofoetal outcomes: a systematic review and meta-analysis. *BMC Pregnancy Childbirth* **20**, 426 (2020). https://doi.org/10.1186/s12884-020-03116-2).
3. Purcell RH, Emerson SU. Hepatitis E: an emerging awareness of an old disease. J Hepatol. 2008;48:494–503.

4. Kamar N, Dalton HR, Abravanel F, Izopet J. Hepatitis E virus infection. Clin Microbiol Rev. 2014 Jan;27(1):116-38. doi: 10.1128/CMR.00057-13. PMID: 24396139; PMCID: PMC3910910.
5. Said B, Ijaz S, Chand MA, Kafatos G, Tedder R, Morgan D. Hepatitis E virus in England and Wales: indigenous infection is associated with the consumption of processed pork products. Epidemiol Infect. 2014;142(7):1467-75. 10.1017/S0950268813002318.
6. Mooij, S.H., Hogema, B.M., Tulen, A.D. *et al.* Risk factors for hepatitis E virus seropositivity in Dutch blood donors. *BMC Infect Dis* **18**, 173 (2018). https://doi.org/10.1186/s12879-018-3078-9.
7. Jilani N, Das BC, Husain SA, Baweja UK, Chattopadhya D, Gupta RK, Sardana S, Kar P. Hepatitis E virus infection and fulminant hepatic failure during pregnancy. J Gastroenterol Hepatol. 2007 May;22(5):676-82. doi: 10.1111/j.1440-1746.2007.04913.x. PMID: 17444855.
8. Kourtis AP, Read JS, Jamieson DJ. Pregnancy and infection. N Engl J Med. 2014 Jun 5;370(23):2211-8. doi: 10.1056/NEJMra1213566. PMID: 24897084; PMCID: PMC4459512.
9. Pérez-Gracia MT, Suay-García B, Mateos-Lindemann ML. Hepatitis E and pregnancy: current state. Rev Med Virol. 2017 May;27(3):e1929. doi: 10.1002/rmv.1929. Epub 2017 Mar 20. PMID: 28318080.
10. Guthmann JP, Klovstad H, Boccia D, Hamid N, Pinoges L, Nizou JY, Tatay M, Diaz F, Moren A, Grais RF, Ciglenecki I, Nicand E, Guerin PJ. A large outbreak of hepatitis E among a displaced population in Darfur, Sudan, 2004: the role of water treatment methods. Clin Infect Dis. 2006 Jun 15;42(12):1685-91. doi: 10.1086/504321. Epub 2006 May 12. PMID: 16705572.
11. Ahmed A, Ali Y, Siddig EE, Hamed J, Mohamed NS, Khairy A, Zinsstag J. Hepatitis E Virus Outbreak among Tigray War Refugees from Ethiopia, Sudan. Emerg Infect Dis. 2022 Aug;28(8):1722-1724. doi: 10.3201/eid2808.220397. PMID: 35876603; PMCID: PMC9328910.
12. Li P, Liu J, Li Y, Su J, Ma Z, Bramer WM, Cao W, de Man RA, Peppelenbosch MP, Pan Q. The global epidemiology of hepatitis E virus infection: A systematic review and meta-analysis. Liver Int. 2020 Jul;40(7):1516-1528. doi: 10.1111/liv.14468. Epub 2020 Apr 24. PMID: 32281721; PMCID: PMC7384095.
13. Shi, Lingfeng, et al. "Increasing positive rate of IgG against hepatitis E virus with steady IgM positivity and clinical incidence: A retrospective seroprevalence time series analysis of HEV from 2012 to 2021 in Chongqing, China." *Journal of Medical Virology* 95.6 (2023): e28872.
14. Ehi Airiohuodion P, Wartel A, Yako AB, Mac PA. Seroprevalence and burden of hepatitis E viral infection among pregnant women in central Nigeria attending antenatal clinic at a Federal Medical Centre in Central Nigeria. Front Med

(Lausanne). 2022 Sep 2;9:888218. doi: 10.3389/fmed.2022.888218. PMID: 36117965; PMCID: PMC9478474.

15. Abebe M, Ali I, Ayele S, Overbo J, Aseffa A, Mihret A (2017) Seroprevalence and risk factors of Hepatitis E Virus infection among pregnant women in Addis Ababa, Ethiopia. PLoS ONE 12(6): e0180078. https://doi.org/10.1371/journal.pone.0180078.
16. Simani OE, Seipone TP, Selabe G, Seheri LM, Mphahlele MJ, Mayaphi SH, Steele AD. Low seroprevalence of hepatitis E virus in pregnant women in an urban area near Pretoria, South Africa. IJID Reg. 2021 Dec 11;2:70-73. doi: 10.1016/j.ijregi.2021.12.002. PMID: 35757069; PMCID: PMC9216388.
17. Capai L, Masse S, Gallian P, Souty C, Isnard C, Blanchon T, Peres B, de Lamballerie X, Charrel R, Falchi A. Seroprevalence Study of Anti-HEV IgG among Different Adult Populations in Corsica, France, 2019. Microorganisms. 2019 Oct 16;7(10):460. doi: 10.3390/microorganisms7100460. PMID: 31623185; PMCID: PMC6843757.
18. Kmush BL, Lu AM, Spillane T, Hruska B, Gump BB, Bendinskas KG. Seroprevalence of hepatitis E virus antibodies in adults and children from upstate New York: A cross-sectional study. PLoS One. 2021 Jan 26;16(1):e0245850. doi: 10.1371/journal.pone.0245850. PMID: 33497387; PMCID: PMC7837474.
19. Lu J, Huang Y, Wang P, Li Q, Li Z, Jiang J, Guo Q, Gui H, Xie Q. Dynamics of Hepatitis E Virus (HEV) Antibodies and Development of a Multifactorial Model To Improve the Diagnosis of HEV Infection in Resource-Limited Settings. J Clin Microbiol. 2021 Jan 21;59(2):e02321-20. doi: 10.1128/JCM.02321-20. PMID: 33239375; PMCID: PMC8111123.
20. Mansuy JM, Bendall R, Legrand-Abravanel F, Sauné K, Miédouge M, Ellis V, Rech H, Destruel F, Kamar N, Dalton HR, Izopet J. Hepatitis E virus antibodies in blood donors, France. Emerg Infect Dis. 2011 Dec;17(12):2309-12. doi: 10.3201/eid1712.110371. PMID: 22172156; PMCID: PMC3311200.
21. Li RC, Ge SX, Li YP, Zheng YJ, Nong Y, Guo QS, Zhang J, Ng MH, Xia NS. Seroprevalence of hepatitis E virus infection, rural southern People's Republic of China. Emerg Infect Dis. 2006 Nov;12(11):1682-8. doi: 10.3201/eid1211.060332. PMID: 17283617; PMCID: PMC3372335.
22. Sharifipour S, Davoodi Rad K. Seroprevalence of hepatitis E virus among different age groups in Tehran, Iran. New Microbes New Infect. 2019 Dec 20;34:100638. doi: 10.1016/j.nmni.2019.100638. PMID: 31993207; PMCID: PMC6976942.
23. Daniel HD, Warier A, Abraham P, Sridharan G. Age-wise exposure rates to hepatitis e virus in a southern Indian patient population without liver disease. Am J Trop Med Hyg. 2004 Nov;71(5):675-8. PMID: 15569803.

24. Wu, Chunchen, Xiaoxue Wu, and Jianbo Xia. "Hepatitis E virus infection during pregnancy." *Virology Journal* 17 (2020): 1-11.
25. Aggarwal R. *The global Prevalence of Hepatitis E virus Infection and Susceptibility: a Systematic Review*. Geneva: World Health Organization; 2010..
26. Qian, Zhongyao, et al. "Prevalence of hepatitis E virus and its association with adverse pregnancy outcomes in pregnant women in China." *Journal of Clinical Virology* 158 (2023): 105353.