Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 13, Issue, 11, pp.12231-12234, November, 2022

RESEARCH ARTICLE

DETERMINATION OF HEPATITIS E VIRUS AND PLATELETS COUNTS AMONG MISCARRIAGE WOMEN IN SAUDI HOSPITAL IN KHARTOUM STATE AT 2019

[1,*]Hanan Malik Ali Abdalla and [2]Dr. Amira Eltom Fawzi Osman

[1]M.SC of Microbiology and Immunology
[2]Department of Microbiology – Faculty of Medical Laboratory science - AlzaiemAlazhari University –Sudan

ARTICLE INFO

Article History:
Received 15th August, 2022
Received in revised form
19th September, 2022
Accepted 24th October, 2022
Published online 30th November, 2022

Keywords:
Hepatitis E virus, Platelets Counts, Immuno Chromatographic Test (ICT), Enzyme-Linked Immunosorbent Assay (ELISA).

ABSTRACT

Hepatitis E virus (HEV) is an important enteric human pathogen worldwide distribution. It can cause sporadic cases as well as large epidemics of acute hepatitis. Many studies proved that HEV infection in pregnancy in the first trimester leads to miscarriage and in the third trimester poor maternal and fetal outcome. Study aimed to determine the prevalence of HEV among miscarriage women attending Saudi Hospital for in Khartoum, during the period from September to December 2019. A total of 70 aborted women .Blood specimens were collected and serum analyzed using Immuno chromatographic test (ICT) IgG and IgM, Enzyme-Linked Immunosorbent Assay (ELISA) for detection of HEV Anti HEV IgM. A total of 70 miscarriage women were included in the study. 5 (7.1%) were positive for anti-HEV IgG and ICT (IgM), while 65 (92.9%) were negative for anti-HEV IgG and ICT (IgM), and were negative for anti-HEV IgM by ELISA, there was insignificant relationship on seroprevalence HEV IgM (positive>1.1).The high prevalence noted among age group 21 – 40years for ICT IgG and IgM. All of the participants (miscarriage women)were analyzed platelets which range from 155 to 432 cell/cubic milliliter.

Citation: ***Hanan Malik Ali Abdalla and Dr. Amira Eltom Fawzi Osman*, 2022.**" Determination of Hepatitis E Virus and Platelets Counts among Miscarriage Women in Saudi Hospital in Khartoum State at 2019", *Asian Journal of Science and Technology*, 13, (11), 12231-12234.

INTRODUCTION

Hepatitis E virus (HEV) is Liver disease cause by hepatitis (Okamoto, 2007), the HEV particles are spherical (Balayan, 1983; Tam, 1991), non- enveloped with diameter of 32-34 nm. They belong to the caliciviride family, although relation to the other member of the family is relatively distant (Purcell, 2008). The capsid protein encoded by the open reading frame 2(ORF2) gene of HEV is the only known structural protein on the virion. HEV is a major cause of hepatitis transmitted by the fecal oral route and it is a common cause of waterborne epidemics of hepatitis in Asia, Africa, India & Mexico but is more common in the United States (Kasper, 2015). HEV is first documented in samples collected during the Delhi outbreak of 1955, when 29.000 cases of icteric hepatitis occurred after sewage contamination of the city's drinking water supply (Brain, 2010). A large outbreak of hepatitis E was reported in June 2004 in the internally displaced population camps of Darfur, in Western Sudan, and across the border in Chad, at least 5000 HEV infections were recorded from June to December 2004 (Guthmann, 2004).

Corresponding author:** ***Hanan Malik Ali Abdalla,
M.SC of Microbiology and Immunology.

The infection primarily occurs in young adults and is generally mild and self-limiting; however, the mortality rate is higher among women, especially during the second or third trimesters of pregnancy (Purcell, 2008; Benait, 2007). The incidence and severity during pregnancy vary widely around the world. In Western Europe and North America, the incidence is as low as one in 20,000, whereas in outbreaks of waterborne Hepatitis E in India and Asia, the case fatality rate is 1–2 % and up to 10–20 % in pregnant women (Brooks, 2010). Reason for the difference in the outcome of HEV in different geographical areas remains unclear (Lindemann, 2010) but could be due to early childhood HEV exposures, producing long-lasting immunity and/or modifying subsequent responses to exposure to the virus. HEV is known to have four genotypes, which have been detected in humans; genotypes 1 and 2 are more virulent, genotypes 3 and 4 are more attenuated and accountable for subclinical infections (Lindemann, 2010). Pregnant women and patients with pre-existing chronic liver diseases at a particular risk of fulminant hepatic failure upon HEV infection (Haaheim, 2002). Pregnancy appears to be a potential risk factor for viral replication and leads extreme low immune status of pregnant women. Mortality rates among pregnant women, especially those infected in the third trimester, have ranged between 5% and 25%, much higher than in men and non pregnant women (Pischke, 2011).

Hepatitis E in pregnancy is also associated with high rates of spontaneous abortion, intrauterine death, and preterm labour (Khuroo, 1981). Estimated one- third of the world's population has been infected with HEV (Balayan, 1983). It is the most or second most common cause of acute viral hepatitis among adults throughout much of Asia, the Middle East, and Africa (Tam et al. 1991; Okamoto, 2007). HEV infection is usually self-limiting, but may develop in to fulminate hepatitis with a case-fatality rate (CFR) between 1 and 2% in the general population. HEV is classically transmitted feco-orally (food borne, waterborne), although person to person transmission has also been reported . HEV has been occasionally linked to nosocomial spread. Vertical transmission from mother to infant is also known to occur. It is infrequently transmitted by transfusion of blood or blood products. Incubation period following exposure to hepatitis E virus is 3-8 weeks with a mean of 40 days. A large outbreak of hepatitis E was reported in June 2004 in the internally displaced population camps of Darfur ,in Western Sudan. Pregnant women and patients with pre-existing chronic liver diseases at a particular risk of fulminant hepatic failure upon HEV infection Pregnancy appears to be a potential risk factor for viral replication and leads extreme low immune status of pregnant women.

MATERIAL AND METHOD

Study design: This was descriptive and cross-sectional study.

Study duration: This study was conducted during the period from September to December 2019.

Study area: The study was conducted in Saudi Hospital in Khartoum.

Study population: All miscarriage women attending in Saudi Hospital the study period were included.

Sample size: Total of 70 miscarriage women were participated in this study.

Data collection: Data collected by direct interviewing questionnaires included age, gestation of miscarriage, history of frequency miscarriage, educational status, and occupational status.

Data analysis: Data was collected and analyzed by a computer system using statistical package for social science (spss).

Ethical consideration: The ethical clearance was obtained from the Ethical Committee Board of Al Zaeim Al Azhari University and permission letter to collect specimen ,Informed consent was obtained from each abortion lady after describing the goal of the study, any favourable outcome and potential risks that might be encountered.

Experimental Work

Collection of Specimen: Three ml of venous blood were collected from each participant under Aseptic condition into sterile plain container 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 -20oC until used.

Specimens processing: Specimens were analyses for HEV IgM and IgG Immuno chromatographic test (ICT)and IgM by Enzyme linked immunosorbent assay (ELISA)**,**(Anti-HEV ELISA IgM, EUROIMMUN Medizinische Labordiagnostika AG,Germany)

Assay principle

Assay method

Immuno chromatographic Test: HEV IgG and IgM Rapid test is a lateral flow chromatographic immunoassay for the qualitative detection of IgG and IgM antibody to HEV in human serum. It was used as screening test. The reactive specimens with the HEV IgG and IgM Rapid test were confirmed with alternative testing method and clinical finding.

Test principle: The HEV IgG and IgM Rapid test is a lateral flow chromatographic immunoassay. The test measure consists of:

- Colored conjugate and pad containing HEV antigens companied with colored gold HEV conjugate.
- Strip containing attest line (T line) and control line (C line)

The T line is pre-coated with monoclonal antihuman IgG and IgM antibody ,and the C line is pre-coated with goat. When an adequate volume of test specimen dispensed in to the sample well of the cassette, the specimen migrates by capillary action across the cassette. Anti-HEV IgG and IgM if present in the specimen will bind to the HEV conjugate .the immunocomplex is then capture on the membrane by the pre-coated anti-human IgG and IgM forming burgundy colored T line , indicating a HEV IgG and IgM positive test result. Absences of the test line suggest a negative result.

Reagent preparation: All reagents are ready to used as supplied. All the specimens were examined for the presence of HEV IgG and IgM antibodies using immunochromatographic immune assays the following steps:

Be sure to label the device with specimen ID number

- The pipette dropper was filled with specimen. holding the dropper vertically, then dispensed 1 drop (about 5 ml) of serum into the well device of ICT card
- Two drops of HEV buffer was added (about 50-70 ml) sample diluents
- The timer was set up
- Results were reading in 15 minutes. Positive results can be visible in as short as 1 minute.

Negative results: If only the C line is developed, the test indicates that no detectable IgG and IgM anti- HEV is present in the specimen. The results is negative or non reactive.

Positive results: If both C and T lines are developed, the test indicates for presence of IgG and IgM anti- HEV in the specimen .the result is positive or reactive.

Enzyme linked immunosorbent assay: The samples were diluted 1:100 with sample buffer.

Principle: This is an ELISA assay for semi-quantitative determination for human antibodies of the IgM in serum or plasma. 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 coated with recombinant antigens of hepatitis E virus. The patients' serum samples added, and during the first incubation step, any IgM class antibodies will be captured in the well. After washing all other substances removed, the specific HEV IgM captured o is then detected by the addition of anti human IgM labeled with enzyme horseradish peroxidase (HRP-conjugate). During second incubation, the Anti human IgM-HRP conjugated 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 HEV IgM 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 which proportional to the amount of antibody captured in the wells, wells negative for HEV IgM remain colorless.

Numbering the wells: The strips needed were set in strip holder and sufficient number of wells including one blank (B), two calibrator (C1, C2), positive control (PC), and negative control (NC) were numbered.

Adding samples: Amount of 100µl of diluted samples, positive controls, negative controls and calibrators were added into their respective wells by using separate disposable pipette tip for each specimen, negative and positive controls to avoid cross contamination, and then mixed by taping the plate gently to avoid over flowing and contamination of adjacent wells in order to fully distribute the samples.

Incubation (1): The plate was covered with plate cover and incubated for 30 minutes at room temperature (18-25oC).

Washing (1): After the end of incubation the plate cover was removed and discarded. The wells were washed manually with 300µl of working strength wash buffer 3 times, wash buffer was left for 30 to 60 seconds per washing cycle, then the wells were emptied.

Adding HRP-conjugate: An amount of 100µl of HRP-conjugate was added into each of the microplate wells.

Incubation (2): The plate was covered and incubated for 30 min at room temperature (18-25oC).

Washing (2): After the end of incubation the plate cover was removed and discarded. The wells were washed with diluted washing buffer 3 times.

Coloring: An amount of 100µl of chromogen/ substrate solution were added into each well. The strips were covered with plate cover and incubated at room temperature for 15 minutes avoiding direct sun light. The enzymatic reaction between the chromogen solutions produced blue color in positive control and anti HEV positive sample wells.

Stop reaction: Amounts of 100µl of stop solution (0.5M Sulphuric acid) were added into each wells and mixed by tapped the plate gently, intensive yellow color developed in positive sample wells.

Measuring the absorbance: Photometric measurement of the color intensity was calibrated with blank well and the absorbance was read at wavelength of 450nm and the reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution.

Interpretation of the result: Each micro plate has been considered separately when calculating and interpreting results of the assay, regardless of the number plates concurrently processed. The results are interpreted as a ratio of the sample OD (450nm) and cut-off value (CO).

Calculation of cut-off value: The extinction value of the calibrator defines the upper limit of the reference range of non infected persons (Cut-off) recommended by kit manufacture (EUROIMMUN) , values above the indicated cut-off are to be considered as positive , those below as negative.

The ratio for each specimen was calculated as follow:

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

Ratio < 0.8: Negative
Ratio ≥ 0.8 to < 1.1: Borderline
Ratio ≥ 1.1: Positive

Negative result: Sample giving absorbance less than the cut-off value are negative for this assay, which indicate that no antibody to hepatitis E virus has been detected with this anti hepatitis E virus ELISA kit. The patient is probably not infected with hepatitis E virus.

Border line: Sample with absorbance OD greater or equal cut-off are considered borderline and retesting of those samples should be taken 7 days later and re-tested in parallel with the first patient. For duplicate determinations the mean of the two values should be taken.

Positive result: Sample giving an absorbance greater than or equal to the cut-off value are considered initially reactive which indicates that antibody to hepatitis E virus have probably been detected using this anti HEV ELISA kit.

RESULTS

A total of 70 aborted women who attended the Saudi Hospital for Obstetrics and Gynecology in Khartoum were enrolled in this study during the period from September to December 2019, to determine serofrequency of Hepatitis E virus, their age ranged from 15 to 44 years old with mean age of (7±22) the majority of aborted women were in the age groups of 21-40 representing 7.1%, all of the participants with normal platelets which range from 155 to 432 cell/cubic milliliter with mean of (274.80±69.11). And to detect relation between the presence of HEV and other factors (age, education, Occupation, Months of aborted, Frequency of aborted).

Table 1. Frequencies of IgM ICT result

IgM ICT	Frequency	Percent%
Positive	2	2.9%
Negative	68	97.1%
Total	70	100%

Table 2. Frequencies of IgG ICT result

IgG ICT	Frequency	Percent%
Positive	3	4.3%
Negative	67	95.7%
Total	70	100%

Table 3. Frequencies of study population according to age groups

Age groups	Frequency	Percent%
up to 20	11	15.7%
21-30	30	42.9%
31-40	27	38.6%
41-50	2	2.9%
Total	70	100%

DISCUSSION

Hepatitis E virus (HEV) cause epidemic, especially in developing countries where hygiene is poor and many affected pregnant women suffer from hepatitis [98]. IgM antibody to HEV in healthy subject has been used to measure the virus which is appears early during acute clinical illness but wanes over a few months, so it detect Acute HEV infection [99]. In this study, a total of 70 miscarriage women who attended Saudi Hospital in Khartoum were enrolled in this study during the period from September to December 2019, to determine serofrequency of HEV, their age ranged from 15 to 44 years old with mean age of (7±22) the majority of aborted women were in the age groups of 21-40 representing 81.4%, all of the participants with normal platelets which range from 155 to 432 cell/cubic milliliter with mean of (274.80±69.11), showed 5(%) seropositivity of ICT as screening(IgG and IgM), this finding agree with that observed in the study conducted among aborted women in Wad Medani Teaching Hospital [100], were confirmed to seropositivy for HEV ELISA using IgM antibody is ingnificant. HEV infection during pregnancy leads to severe complications which may result in fetal and/or maternal mortality, abortion, premature delivery, or death of a live-born baby soon after birth. In Sudan, a fatality rate of 17.8% was found during an outbreak in Darfur, with a rate of 31.1% among pregnant women [99], also another study in Khartoum State conducted by Alngashi, 2014 revealed higher rates of HEV infection (14.5%), however the present study result was higher than that which obtained by Walla, 2014 study also in Khartoum state, showed that (3.3%) of pregnant women were seropositive for anti-HEV IgM. The reason for caring out this study is a very high risk of vertical transmission of HEV from the mother to the fetus which may associate with miscarriage, stillbirth, or neonatal death in of infants.

Limitation

- This study was small sample size.
- The use of confirm techniques for diagnosis of by PCR.
- This study was a little will period

REFERENCES

Balayan , M . S . A . C . Andjaparidze, S . S . Savinskaya , E . S . Ketiladze , D . M . Bragingsky , A . P . Savinov , and V. E . Poleschuk . Evidence for a virus in non-A , non- B hepatitis transmitted via the fecal- oral route . Intervirology . 1983;20:23-31 .

Tam , A . W . M .M . Smith , M . E . Guerra , C .C . Huang, D . W . Bradley , K . E . Fry . and G . R . Reyes . Hepatitis E virus (HEV) : Molecular cloning and sequencing of the full – length viral genome . Virology . 1991;185;120 – 131 .

Okamoto , H. Genetic variability and evolution of hepatitis E virus . Virus Res . 2007 ; 127:216 –228 .

Purcell RH, Emerson SU. Hepatitis E: an emerging awareness of an old disease. J Hepatol. 2008;48:494–503.

Benait VS, Sander V, Purikh F, et al. Outcome of acute hepatic failure due to acute Hepatitis E in pregnant women. Indian J Gastroenterol. 2007;26:6–10.

Kasper L, Fauci J. Acute viral hepatitis. Harrison's Princ Intern Med. 2015;2(18):2537–55.

Lindemann ML, Gabilondo G, Romero B, et al. Low prevalence of Hepatitis E infection among pregnant women in Madrid, Spain. J Med Virol. 2010;82:1666–8.

Brain WJ and Marc HV. Hepatitis E virus. In: Desk Encyclopedia of Human and medical virology 11th edition Elsevier USA 2010;PP:195-198.

Brooks GF, caaroll KC Butel Js , Morse SA and Mietzer TA. Hepatitis ;2010

Guthmann JP, klovstad H, Boccia D, Hamid n, Pinoges L, Nizou J.Y, *et al*. A large outbreak of hepatitis E among a displaced population in Darfur, Sudan ,2004: The role of water treatment methods. *Clin Infect Dis*. 2006;421685-1691.

Haaheim LR ,Pattison JR and Whitley RJ. Hepatitis E virus .In :A practical guide to clinical virology, second edition ,JoHN WILEY & SONS , LTD , England. 2002;PP 125-198.

Pischke S, Heim A and Bremer B. Hepatitis E: an emerging infectious disease in Germany?. *Z gastroenterol* .2011 ;49:1255-1257

Khuroo MS, Teli MR and Skidmore S. Sofi MA, Khuroo MI. Incidence and severity of viral hepatitis in pregnancy. *Am J Med*. 1981;70: 252-255.

****