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Seroprevalence of Subclinical Hepatitis E in Pregnancy

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ABSTRACT

CONTEXT: Prevalence of Hepatitis E in developing countries ranges from 7.2% to 24.5%Case fatality rate in pregnancy-25%. Diagnosis of HEV infection remains challenging.and HEV vaccines are under trial. Hence by assessing the seroprevalence of HEV in pregnant mothers, vaccination can implemented, to protect asymptomatic women with subclinical infection from fulminant outcome.

AIM & OBJECTIVE: To study the prevalence of HEV infection in pregnant mothers attending antenatal clinic in our hospital in one year.

MATERIALS & METHODS: IgM, IgG antibodies to HEV & Viral RNA were detected by ELISA and RT PCR respectively and risk factors were evaluated with a questionnaire.

RESULTS: 4.5% of IgM & 6.5% of IgG is the seroprevalence in asymptomatic pregnant women associated with poor sanitation, inadequate drainage facilities and non-vegetarian diet..

CONCLUSION:Despite low prevalence of subclinical HEV infection, effective vaccination & public health awareness, helps fight against fulminant hepatic failure.

KEYWORDS: Seroprevalence, ELISA, RT PCR.

INTRODUCTION:

Hepatitis is a condition characterised by the presence of inflammatory cells in the liver tissue. Hepatitis A & E are transmitted through faeco-oral route; Hepatitis B & C are transmitted through blood and body fluids. Hepatitis E causes large outbreaks in endemic areas like India, Central Asia, parts of Africa & Mexico. HEV infection is epidemic in countries with suboptimal sanitary conditions.

HEV causes an acute, self-limiting viral hepatitis, typically lasting for 1- 4 weeks. HEV infection has grave prognosis during pregnancy leading to fulminant hepatic failure with risk of DIC. 37% of viral hepatitis during pregnancy is caused by Hepatitis E & 81% of them go in for fulminant hepatic failure during 3rd trimester. Case fatality rate among pregnant mother is 25%. HEV infection in pregnancy has high risk of malformation, abortion, still birth & neonatal death.

Most of the cases with HEV infection remains asymptomatic. Acute infection is treated with supportive care. Laboratory diagnosis of Hepatitis E is based either on serology or NAA technique.

Candidate vaccines against HEV are on trial, very effective & accepted in China, showing efficacy of >90%. HEV is accountable for approximately 9.8% of pregnancy associated deaths. In southern Asia as many as 10,500 maternal deaths per year could be prevented by using the existing vaccine.

As there is very little data documents pertaining to sero-prevalence of Hepatitis E in pregnant mother in India, this study was done to assess the sero-prevalence of subclinical Hepatitis E viral infection in asymptomatic pregnant mother attending routine AN check up in a tertiary care hospital in Chennai.

Since Hepatitis E infection is a world health problem, there is a need for more public health involvement by provision of Clean drinking water, Health education of public & easy availability of approved serological assay for early detection of infection.

MATERIALS AND METHODS:.

TYPE OF STUDY:.	Cross-sectional study
STUDY PLACE:.	Department of Obstetrics & Gynaecology, Department of Microbiology & Medical Gastroenterology, SMCH, Chennai.
PERIOD OF STUDY:.	May 2015- September 2015.
SAMPLE SIZE:.	200
INCLUSION CRITERIA:.	All asymptomatic pregnant mothers age >18 years.
EXCLUSION CRITERIA:.	Alcohol intake, Chronic drug intake, Past history of jaundice, Chronic medical illness.

PATIENT SELECTION:.

Study was explained to pregnant mothers in their local language & informed consent was obtained. 200 mothers were recruited, blood samples were collected, serum separated & the presence of IgM, IgG antibodies to HEV & Viral RNA was detected by serology & PCR respectively.

ETHICAL CONSIDERATION:.

The study was approved by College Ethical Committee.

STATISTICAL ANALYSIS:.

The collected data was analysed with Stata 12 version. Chi-square test was used to calculate the significance. The probability value of 0.05 was considered significant.

DATA COLLECTION:.

Details were obtained with the help of a questionnaire containing age, residential address, educational status, profession, SES, source of drinking water & type of toilet facility to analyse various factors that affect the prevalence of HEV infection in pregnant mothers.

SAMPLE COLLECTION;

5ml of blood was drawn from each individual & serum separated by cetrifugation at 2500 rpm for five minutes. Samples were duplicated and stored in 2ml cryo-vials containing 50 microlitres of EDTA at -80°C deep freezer until tested.

ELISA:. ELISA was done with a commercial kit. DSI- EIA- ANTI-HEV- IgM- KIT

DSI- EIA- ANTI-HEV- IgG- KIT.

PRINCIPLE:.
The microtitre test wells are coated with HEV antigens. Diluted patients serum are added to their designated wells. If specific Abs to the Ag are present in the patients serum, they bind to each other in the wells during the incubation period. Test wells are washed & unbound Abs are removed. To the microtitre plate, freshly prepared enzyme conjugate is added. Enzyme conjugate specifically binds to antigen-antibody complex and a chromogen substrate is added. The peroxidase enzyme present in the substrate will catalyse the reaction & turns the chromogen to blue colour. Addition of stop solution at the end of the reaction turns blue colour to a bright yellow colour.

The reaction is read with ELISA reader.
PREPARATION OF VIRAL NUCLEIC ACID:.
PRINCIPLE:.
Cells are lysed during a short incubation period with chaotropic salt; which immediately inactivate all nucleases. Cellular nucleic acids bind selectively to special glass fibres pre-packed in purification filter tubes. Bound nucleic acids are purified in a series of rapid 'wash and spin' steps to remove contaminating cellular components. The spin column is centrifuged at 1200 rpm for 2 min & elution buffer is added. The spin column is again centrifuged and the eluted nucleic acid is stored at -80°C for later analysis by Seminested PCR in two steps.

1. cDNA synthesis 2.PCR reaction setup
OBSERVATION & RESULTS:.
200 asymptomatic pregnant women were included in the study.

TABLE 1:.AGE DISTRIBUTION:.

AGE IN YEARS	PERCENTAGE DISTRIBUTION
<20	13%
21-30	82%
>30	5%

TABLE 2:.TRIMESTER DISTRIBUTION:.

TRIMESTER	PERCENTAGE DISTRIBUTION
1st	12%
2nd	49%
3rd	39%

TABLE 3:.PARITY DISTRIBUTION;.

GRAIVDA	PERCENTAGE DISTRIBUTION
Primi	55%
Multi	45%

TABLE 4:.EDUCATIONAL STATUS:.

EDUCATIONAL STATUS	PERCENTAGE DISTRIBUTION
Illiterate	7%
Elementary	10%
High school	33%
Higher secondary	40%
Graduate	10%

TABLE 5:.SOCIOECONOMIC STATUS(Modified Kuppusamy Socioeconomic status Scale 2015)

SOCIOECONOMIC STATUS	PERCENTAGE DISTRIBUTION
Upper	0%
Upper middle	9%
Lower middle	29%
Upper lower	57%
Lower	5%

TABLE 6:.SOURCE OF DRINKING WATER:.

SOURCE	PERCENTAGE DISTRIBUTION
Metro water	61.5%
Bore well water	14%
Purified can water	25%

TABLE 7:.DRAINAGE FACILITIES AT HOME:.

DRAINAGE FACILITIES	PERCENTAGE DISTRIBUTION
Adequate	25%
Inadequate	75%

TABLE 8:.SANITARY HABITS;.(Habit of hand washing before food & after Using toilet)

SANITARY HABITS	PERCENTAGE DISTRIBUTION
Good	79%
Poor	21%

TABLE 9:. DIETARY HABITS:.

DIETARY HABITS	PERCENTAGE DISTRIBUTION
Vegetarian	54%
Non-vegetarian	46%

Result of IgM & IgG ELISA were analysed with respect to possible risk factors of HEV disease.

IgM positive by ELISA:. 9
21-30 years age - 9/9
Primigravida- 4/9
Multigravida- 5/9
Trimester distribution- Equal
Non- vegetarian- 7/9 (p = 0.04)
Poor sanitary habits- 8/9 (p < 0.05)
Metro water- 7/9 (p <0.05)
Inadequate drainage facility – 7/9 (p <0.05)
Illiterate - 7/9 (p < 0.05)
Upper lower class - 5/9 (p < 0.05)

IgG positive by ELISA:. 13
21-30 years age - 13/13
Primigravida- 7/13
Multigravida- 6/13
Trimester distribution- 9/13 (p = 0.05)
Non- vegetarian- 9/13 (p = 0.07)
Poor sanitary habits- 9/13 (p < 0.05)
Metro water- 8/13 (p <0.05)
Inadequate drainage facility – 6/13 (p = 0.06)
Upper lower class - 6/13 (p < 0.05)

DISCUSSION;.
Earlier to ELISA, HEV was diagnosed by excluding HAV & HBV (NANB Virus). ELISA for detection of HEV antibodies IgM & IgG is highly sensitive and inexpensive. RT- PCR is a nucleic acid amplification technique. The test is of use in window period when serological tests are negative. It helps in detecting specific HEV RNA in blood & faeces.

AGE DISTRIBUTION:.
Study by shams et al- mean age was 25 year. Study by Nargis Begum et al- mean age was 21.92 + 2.6 years. In our study, mean age of distribution was 24.12 years, the predominant population group belonged to this age.

GESTATIONAL AGE:.
According Nargis Begum et al, mean period of gestation was 19.06 + 2.2 weeks. In our study most cases were in second & third trimesters.

EDUCATIONAL STATUS;.
Educational status affects the prevalence of subclinical infection significantly as the illiterate population was prone to develop infection. Study done by Sekan et al also showed educational status to be a significant risk factor.

SOCIOECONOMIC STATUS:.
mothers belonged to upper lower class in contrary to study by Nargis et al which showed high prevalence in lower SES. In our study only 5% of mothers belong to this group.

DIET:.
Mothers consuming Non-vegetarian diet were seropositive. This high prevalence is due to consumption of under cooked meat. According to Rakesh et al, HEV RNA has been detected in domestic swine faeces & HEV antibodies in sera of cattle, sheep, pigs and rodents.

ANALYSIS OF HEV SEROPOSITIVITY:.
4.5% (9/200) IgM & 6.5% (13/200) IgG was the seroprevalence rate in asymptomatic pregnant women. According to Daniel et al - study in 600 samples from Vellore (2014) - IgG HEV prevalence was 5.62%. Studies form North India – showed IgG prevalence of 33.67% in pregnant mothers.

CONCLUSION:.
According to socio-demographic characteristics with Hepatitis E seropositivity in asymptomatic pregnant women, we found that HEV exposure was positively associated with inadequate drainage, poor sanitation, non-vegetarian diet. Low educational status may be related with poor hygiene & low SES associated with poor toilet practices. Analysis of Anti-HEV seroprevalence with age, gravida & gestational age revealed no significant association.

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