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Association of hepatitis E seropositivity and altered progesterone levels in pregnant women of low socioeconomic status from capital region of Pakistan

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Abstract

Objectives: To investigate the seroprevalence of hepatitis E virus infection, risk factors and its association with progesterone levels in pregnant women from low socioeconomic background.

Methods: The cross-sectional study was conducted in Rawalpindi and Islamabad, Pakistan, from January to July 2012, and comprised pregnant asymptomatic healthy females from different clinics and hospitals of the twin cities. Data was collected using a predesigned demographic questionnaire to determine socioeconomic status. Prevalence of anti-hepatitis E virus antibodies and progesterone levels were determined using enzyme-linked immunosorbent assay kits.

Results: Of the 90 women, 35(39%) were in the 21-25 year age group, and 55(61%) belonged to low socioeconomic background. The overall prevalence of seropositive hepatitis E virus immunoglobulin-G was 54(60%) and immunoglobulin-M was 12(13.3%). In the first trimester, the levels of

progesterone were higher in patients positive for immunoglobulin-M compared to immunoglobulin-G ($p<0.001$).

Conclusions: Low socioeconomic status appeared to be a potential risk factor associated with high hepatitis E virus seroprevalence and alterations in the normal progesterone levels during pregnancy.

Key Words: Hepatitis E virus, HEV, Progesterone, Seroprevalence, Pakistan.

Introduction

Hepatitis E, caused by hepatitis E virus (HEV), is an infectious viral disease with clinical and morphological features of acute hepatitis. Although it has been reported throughout the world, it is the cause of major outbreaks of waterborne hepatitis in Asia and Africa.[1-2] In the Indian subcontinent, an epidemiological study tracking hepatitis infection reported HEV as responsible for 68% of sporadic hepatitis infections and fulminant hepatic failure in areas of poor sanitary conditions where the virus was endemic.[3] South Asia is endemic for HEV and it accounts for over 50% cases of acute viral hepatitis in endemic countries.[4-5] Acute infection sometimes leads to more severe clinical fulminant hepatic failure in pregnant women and is associated with very high mortality, particularly during the third trimester in endemic areas, whereas it occurs sporadically and is more often food-borne than water-borne in developed countries.[6]

The prevalence of anti-HEV antibodies in healthy populations has been studied in various populations worldwide to measure the extent of exposure to HEV, and it was found that anti-HEV antibodies were present in persons living in all geographical areas, but in disease-endemic areas, the prevalence rates among healthy populations were much higher than those in non-endemic areas.[7]

In endemic areas, detection of immunoglobulin-M (IgM) anti-HEV suggests acute infection, whereas IgG anti-HEV indicates past exposure to the virus.[8] In response to the viral infection, both IgM and IgG antibodies are detected soon

after, with peak antibody titers occurring 2-4 weeks after infection.[9] The persistence of IgG anti-HEV in populations is still not yet established. Large hepatitis E epidemics have been reported among adults in disease-endemic areas, suggesting either that anti-HEV antibody may not be fully protective or that antibody levels decline with time and gradually reach unprotected levels.[7]

It is well established that IgM antibody to HEV appears faster during the infection and disappears in about four months compared to IgG that can persist for more than 10 years.[10]

In pregnancy, normal level of sex steroid hormones and immunity is altered. Hepatitis E infection in pregnancy is associated with high rates of spontaneous abortion, intrauterine death, and preterm labour.[11] The incidence of HEV infection during the second and third trimesters of pregnancy is much higher than in the first trimester that may cause fulminant hepatic failure in more than 30% patients.[5]

Progesterone is critical for the establishment and maintenance of pregnancy, both for its endocrine and immunological effects. Progesterone receptors have been proposed to play a key role in human gestation, maintenance of human labour and parturition.[12] The levels of oestrogens, progesterone and beta- human chorionic gonadotropin (HCG) are often higher in HEV-positive patients compared to HEV-negative patients or control healthy pregnant females.[13] It has also been proposed that elevated levels of sex steroid hormones in women with HEV-associated acute liver failure are a risk factor predisposing women to poorer outcomes.[9]

The current study was planned to determine the seroprevalence of HEV infection in pregnant women from low socioeconomic background, and to identify possible risk factors of HEV infection in relation to socio-demographic factors. It ws also planned to determine the levels of progesterone in HEV-positive pregnant women and compare them with HEV-negative population to determine the role of HEV in alteration of pregnancy-related hormone.

Subjects and Methods

The cross-sectional study was conducted in Rawalpindi and Islamabad, Pakistan, from January to July 2012. After approval from the institutional ethics review board of the National University of Science and technology (NUST), Islamabad, the sample was raised from among pregnant asymptomatic healthy females of low socioeconomic status (SES) who came for routine checkup at different clinics and hospitals of the twin cities.

Data was collected after taking informed consent from the subjects. Those who refused to participate were excluded. The participants were asked to fill out a questionnaire on socio-demographic characteristics, including age, trimester, gravida, family income, disease, place of previous delivery, and contact number. Age was divided into four groups; 16-20, 21-25, 26-30 and 31-40 years. the subjects were divided into primarigravida and multigravida groups, and the later was further divided on the basis of place of previous delivery into three categories of home, government hospital and private hospital. The sample population was divided into lower and relatively higher income groups based on the cut-off value of Pakistan Rupees (PKR) 10,000 per month with the lower income group earning <PKR10,000. Becton, Dickinson (BD) syringes were used to collect 5ml of blood samples in ethylenediaminetetraacetic acid (EDTA) tubes till serum extraction. Serum was extracted from the blood samples by centrifugation at 10,000rpm for 5 minutes. The extracted serum was stored at -80ºC.

For the detection of anti-HEV IgG and IgM, MicroLISA commercial kits (Amgenix, San Jose, CA, USA) were used for specific detection of anti-HEV IgG and IgM antibodies in human sera following the manufacturer's instructions. Enzyme-linked immunosorbent assay (ELISA) readings were read on Biotek Elx800 (Winooski, VT, USA).

The levels of progesterone was assayed using commercially available quantitative RIA kit (AmgenixMicroLISA™ Progesterone Test) (San Jose, CA, USA) and its assay protocol.

For the detection of HBV and HCV, AmgenixOnSight™ hepatitis B surface antigen (HBsAg) test and AmgenixOnSight™ HCV Test (San Jose, CA, USA) were performed as per the manufacturer's instructions.

Data, expressed as frequencies and percentages, was analysed on Microsoft Excel, using one-way analysis of variance (ANOVA), unpaired T test, and one-tailed Pearson correlation test. The level of significance was set at $p<0.05$. GraphPad Prism was used for developing graphs.

Results

Of the 90 women, 35(39%) were in the 21-25 year age group, and 55(61%) belonged to low socioeconomic background. The overall prevalence of seropositive HEV IgG was 54(60%) and IgM was 12(13.3%). The prevalence of IgG and IgM were determined in three trimesters of pregnancy that varied from 21/36 (58.3%) in 1st trimester to 12/19 (63.1%) in the 3rd trimester for IgG, and 2/36 (5.5%) in the 1st trimester to 4/19 (11%) in the 3rd trimester for IgM.

There were 42(46.6%) primigravidae and 48(53.3%) multigravidae. Among the former, 23(54.75 %) were positive for IgG compared to 31(64.5%) among the latter. IgM serpositivity was observed in 5(11.9%) primigravidae and 7 (14.5%) multigravidae Seroprevalence data showed variation related to place of previous delivery for both IgG and IgM (Table 1).

In immunochromatography, of the 54 HEV-positive samples, 4(7%) were also positive for HCV, but none was found positive for HBV.

Patients in the 1st trimester showed higher progesterone levels in IgG-positive cases compared to IgG-negative population (Figure 1A). In the 2nd trimester, IgG-positive patients showed lower progesterone levels compared to HEV-

negative patients (Figure 1B). In HEV-positive pregnant women of third trimester, the levels were low compared to the healthy population (Figure 1C).

In HEV-IgG negative cases, the increase in hormonal levels were according to the normal increase during all three trimesters (Figure 1D) compared to IgG-positive patients in whom increased levels were detected in 1st trimester and lower levels in 2nd and 3rd trimesters (Figure 1E).

Comparison of progesterone levels of HEV IgM and HEV IgG indicated that the levels were significantly higher in case of acute infection compared to past infection (Figure 1F).

Discussion

HEV is prevalent particularly in the developing countries where hygiene conditions are poor and many affected pregnant women suffer from fulminant hepatitis. In the developed countries, a small proportion of population has circulatory antibodies to HEV, whereas in endemic regions, like Pakistan and India, seroprevalence rates are generally higher with considerable variations between regions.[14] The present study reveals a seroprevalence of anti-HEV IgG in healthy pregnant women with low SES to be as high as 60%. This rate is much higher than that reported from previous studies done in Sargodha (16%) and Karachi (20%).[15] Although our findings of 13.3% IgM seropositivity seemed lower than previously reported 20% from urban population of Sindh[16], a recent multicenter study reported a comparable IgM seroprevalence of 15.5% among pregnant women from various regions of Pakistan.[17]

When compared within the region, HEV IgG seroprevalence was found higher than India, where 33% pregnant women and 40-50% adult population was reported seropositive.[18-19] Reports from Bangladeshi pregnant women indicated similar higher patterns with 37.6% HEV IgG seropreprevalence.[20]

The high prevalence of antibodies can be attributed to the sanitation conditions in Pakistan. It is estimated that only 42% of Pakistani population (65% of urban

and just 30% of rural areas) has access to proper sanitation facilities in Pakistan.[21] In urban areas, people live in thickly-populated colonies where open drains, dumping of waste in open places and mixing of drinking and wastewater due to close proximity of drinking and waste water lines is common. A study involving samples taken from different location of drainage outlets of Islamabad and Rawalpindi showed high percentage of 40.7% of HEV circulation. Alternatively, the predominant HEV genotypes in the developed countries could be less virulent than those in developing countries. The HEV genotypes circulating in endemic areas, including Pakistan, are mostly genotypes 1 and 2, which are very uncommon in industrialised countries.[22]

The current study showed a general increase in IgG and IgM prevalence with increasing age, indicating the possibility of re-infection in IgM-positive population. A gradual loss of IgG antibodies over time is an already established phenomenon and antibody levels can fall to critically low levels, resulting in re-infection upon re-exposure to virus.[23] Thus, seroprevalence studies could potentially underestimate the exposure to HEV in a population when only IgG seroprevalence is considered.

A gradual increase in the presence of IgG was seen across the three trimesters of pregnancy. However, in case of IgM, the population of third trimester showed seropositivity of 11% compared to 5.5% in the first trimester. IgG prevalence ratio was higher in multigravida than that in primigravidae group. The possible reason for this trend could be the age of the subjects, which was generally higher in case of multiple pregnancies compared to primigravidae condition. This, in turn, leads to higher chances of infection and re-infection in the population. High incidence of IgG and IgM seropositivity for previous deliveries at home compared to those done in private hospital were related to low SES of the subjects, as reflected in the place where the previous delivery had occurred.

Presence of HEV infection has been associated with high levels of progesterone, ultimately leading to abortion and fulminant hepatic failure (FHF). The normal range of progesterone is critical for the establishment and the maintenance of safe pregnancy, both because of its endocrine and immunological effects. We determined the progesterone levels in the sample population, and surprisingly found that these levels in HEV IgG and IgM-positive population were altered compared to their corresponding seronegative population. Seronegative women in their first trimester were having normal range compared to their IgG-positive counterparts. The results of the second and third trimesters were, however, the opposite, showing decreased progesterone levels for IgG-positive subjects.

These findings are contrary to a study that reported increased level of progesterone in later trimesters, albeit irrespective of disease status. However, HCG and prolactin levels were reported high in HEV-infected women during their first trimesters.[24] Altered progesterone levels in higher trimester could also be a marker of miscarriage as indicated by a previous report.[25] In case of IgM positive samples, the levels were found to be higher than normal in all three trimesters that satisfies the previous reports about increasing progesterone level with the increasing pregnancy length.[24] However, a big dataset may be warranted for significant finding.

This disturbance in the progesterone levels could be due to viral factors, including nutritional deficiency, super infection and folate deficiency. Further investigations, like determination of levels of other pregnancy-related hormones, including oestrogen and beta-HCG, reverse transcription polymerase chain reaction (RT-PCR) in HEV IgG-positive patients, liver function test (LFT), levels of interleukin-10 (IL-10) and IL-12 and their ratio in HEV-positive patients to understand the possible mechanism, need to be done. Association of malnutrition, climate, emotional or physical stress with progesterone deficiency is warranted in seropositive subjects possibly through case-control studies to establish the role of HEV in alteration of progesterone

levels. Furthermore, the role of local genotypic variations, environmental conditions and immunity also needs to be determined.

Three HEV IgG-positive samples, which were also positive for HCV showed abnormally low levels of progesterone. This might be attributed to the possibility that super infection with HCV had further aggravated the disturbance in the hormone levels. However, further studies are recommended in this regard.

The current study also compared the progesterone levels in HEV recent infection and previous infection in females of first trimester. In case of recent infection, the levels of progesterone were high in all samples compared to the levels of previous infection of HEV. This can be compared to report from India wherein alarmingly high levels of hormone were seen in FHF patients.[21]

However, since our study has a limitation of old data, an extended study on Pakistani population may highlight important risk factors associated with HEV infection, pregnancy outcome of infected pregnant females and the identification of alternate transmission pathways. Moreover, the circulating strains of the virus and their virulence potential need to be explored to determine the need of vaccination in pregnant women.

Conclusion

Low socioeconomic status appeared to be a potential risk factor associated with high hepatitis E virus seroprevalence and alterations in the normal progesterone levels during pregnancy.

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Table: Seroprevalence on the basis of age, trimester, gravida, place of delivery and monthly income.

	Total Count (n=90)	IgG Prevalence		IgM Prevalence	
		Count	Percentage	Count	Percentage
Age (years)					
16-20	32	17	53.1	2	6.2
21-25	35	22	62.8	7	20
26-30	15	8	53.3	2	13.3
31-40	8	7	87.5	1	12.5
Trimester					
1	36	21	58.3	2	5.5

2	35	21	60	5	17.1
3	19	12	63.1	4	11
Gravida					
Primigravida	42	23	54.7	5	11.9
Multigravida	48	31	64.5	7	14.5
Previous Delivery Place					
Home	25	18	72	5	20
Govt. Hospital	7	5	71.4	Nil	Nil
Private Hospital	16	7	43.7	1	6.2
Income Groups					
Low	55	38	69	10	18.1
High	35	14	40	3	8.5

Figure: Progesterone levels (ng/ml) in immunoglobulin-G (IgG) seropositive patient population are significantly higher ($p<0.0001$) than that in IgG negative population among (A) first trimester, (B) second trimester ($p<0.0005$) and (C) third trimester ($p<0.0001$). (D) Normal progesterone levels during 1st, 2nd and 3rd trimester in seronegative (IgG-negative) patients in comparison with (E) seropositive altered progesterone levels ($p<0.0005$). (F) Progesterone levels for recent infection denoted by immunoglobulin-< (IgM) seropositivity was significantly higher ($p<0.0002$) than that in past infection denoted by IgG seropositivity.