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Association of Alu I polymorphism in estrogen receptor beta gene with adverse pregnancy outcome in HEV infection

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Abstract: Hepatitis E has both a high incidence and severe course in pregnant women in some geographic regions of HEV (Hepatitis E virus) endemic countries. Intrauterine fetal death, preterm delivery, and perinatal mortality are reported to be higher in pregnant women with HEV infection. Alteration in the steroid hormone levels show high incidence of FHF (Fulminant Hepatic Failure) with high mortality in hepatitis E during pregnancy. The present study is designed to look for the association between ESR (Estrogen Receptor)-beta gene polymorphism for Alu I restriction site and pregnancy outcome. The study group comprised of 142 pregnant women with HEV infection in third trimester, 103 AVH (Acute Viral Hepatitis) cases and 39 ALF (Acute Liver Failure) cases. The control group comprised of 142, age and gestation matched healthy pregnant women with no obstetrics and medical complications. The inclusion criteria for the study group are pregnant women in third trimester with positivity to HEV IgM and/or HEV RNA in the age group of 18-40 years. Genomic DNA is extracted from peripheral blood leukocytes using DNA extraction kit according to the manufacturer's instructions. The polymorphism study is done by using ESR2 specific primers and its genotype is determined by Alu I restriction enzyme. The occurrence of variant A allele for AluI restriction site is significantly higher in mothers with HEV infection who had preterm (25%) than full term delivery (10%) with OR 2.989 (95% CI = 1.265-8.084, $p<0.05$) and low birth weight (26.6%) than average birth weight (6.3%) babies with OR 5.399 (95% CI= 2.01-18.26, $p<0.05$) in pregnant women with HEV infection. The occurrence of variant A allele of ESR beta for AluI restriction site is significantly higher in mother of low birth weight babies (23%) than average birth weight babies (5.5%) in AVH group with OR 5.056 (95% CI= 1.634-21.57, $p<0.05$) and preterm (40.5%) than full term (0%) delivery in ALF group ($p= 0.04$). The higher occurrence of variant A allele for AluI restriction site of ESR-beta gene polymorphism is found to be associated with preterm delivery and low birth weight in pregnant women with HEV infection, preterm delivery in ALF group and low birth weight babies in AVH group.

Key words: HEV infection; third trimester; ESR beta; gene polymorphism; preterm delivery

Introduction

Hepatitis E infection during pregnancy and in the third trimester, especially with genotype 1, is associated with more severe infection and can lead to fulminant hepatic failure and maternal death [1-3]. Hepatitis E has both a high incidence and severe course in pregnant women in some geographic regions of HEV endemic countries, such as Northern India [4]. Intrauterine fetal death, preterm delivery, and perinatal mortality are reported to be higher in pregnant women with HEV infection [4]. The mortality in the second trimester is around 20% and reaches up to 45% in the third trimester [5].

Hormonal factors during pregnancy play a significant role in altering immune regulation or viral replication. The levels of progesterone, estrogen and human chorionic gonadotropin (HCG) increase with pregnancy. Alteration in the steroid hormone levels show high incidence of FHF with high mortality in hepatitis E during pregnancy [6].

HEV-infected pregnant women with FHF had lower CD4 counts and higher CD8 counts and the levels of estrogen, progesterone and β-HCG to be significantly higher in the above-mentioned group when compared with HEV-negative patients or control healthy pregnant females [7]. Although the levels of hormones are physiologically high in the normal control population, patients with HEV infection seemed to have significantly higher levels than controls, which probably explain the direct interaction of HEV with the immune system [7].

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PROGINS carriers (mutation) and reduced expressions of progesterone receptor (PR) and PIBF, a higher IL-12/IL-10 ratio, and a high viral load result in poor pregnancy outcome in Hepatitis E. PROGINS, a haplotype of progesterone receptor is associated with reduced amounts of gene transcript and a lesser response to progesterone; was predominant in FHF compared to AVH (p=0.26) and showed reduced mRNA and protein expression. The risk of fetal mortality in AVH was two times higher (OR, 2.190; CI, 0.303-15.85) and maternal and fetal mortalities in FHF were 4-fold (OR, 4.0; CI, 0.363-44.113) increased in PROGINS carriers, PR and PIBF expressions were lower in AVH and even in lower FHF compared to healthy controls [8].

Hypermethylation of the ERβ promoter is associated with a marked decrease of ERβ mRNA expression in breast cancer, prostate cancer as well as in cancer cell lines, and the inhibition of DNA-methyltransferases reactivates ERβ expression in these cell lines [9-12]. The finding of ERβ gene silencing via promoter hypermethylation in tumors suggests an important role for the ERβ gene in cancer progression and may be used as a prognostic molecular biomarker. Interestingly, a significant increase of ERβ promoter methylation was reported as an epigenetic change in atherosclerosis and vascular aging [13]. Moreover, methylation of a CpG island at the ERβ promoter region showed a primary mechanism responsible for differential expression of ERβ in endometriosis and endometrium [14]. Therefore, keeping in mind the role of progesterone receptor in HEV infection and higher level of estrogen in HEV infection, the present study is designed to look for the association between ESR-beta gene polymorphism for Alu I restriction site and pregnancy outcome.

Materials and Methods

The present study is a prospective case-control study, conducted at the Department of Obstetrics & Gynecology and Department of Medicine, Maulana Azad Medical College and LokNayak Hospital, New Delhi. The sample size is calculated using online Rao Soft sample size calculator at 95% confidence level and assuming 98% of power taking 43.7% of mean prevalence of AVH pregnant out of which 20% infected with HEV, utilizing the data from the Lok Nayak Hospital.

The study group comprised of 142 pregnant women with HEV infection in third trimester, 103 AVH cases and 39 ALF cases. The control group comprised of 142, age and gestation matched healthy pregnant women with no obstetrics and medical complications. The inclusion criteria for the study group are pregnant women in third trimester with positivity to HEV IgM and/or HEV RNA in the age group of 18-40 years. Patients with viral co-infections like HIV, HBV, HCV and HAV, other associated diseases and history of pre-existing liver disease are excluded from the study. The study is approved by the Institutional Ethics Committee, Maulana Azad Medical College, New Delhi.

Two ml of peripheral venous blood is drawn from all the subjects after taking written informed consent and collected in an EDTA tube. Genomic DNA is extracted from peripheral blood leukocytes using DNA extraction kit (Quaigen, USA) according to the manufacturer's instructions. The ESR2 polymorphism study is done by using primers for exon 8 outer primer: GGTTTAGGGGTGGGGTAGACTG and inner primer: CAAGCCTGCCATCACCAAATGAG and the following temperature profile is used 95°C for 15 min for initial denaturation, followed by 35 repeated cycles 30 sec at 94°C for DNA denaturation, 45 sec at 57°C for primer annealing and 45 sec at 72°C for extension, followed by final extension for 7 min at 72°C to get an amplicon of 420bp. The product is digested with AluI restriction enzyme for 1730 G-A polymorphism at 37°C for 150 minutes. The products were visualized in 3% Nusieve agarose gel stained by ethidium bromide under an UV transilluminator. One band of 420 bp is seen for the homozygous G/G samples, three bands of 420, 336 and 84 bp, respectively, for the heterozygous G/A samples, and two bands of 336 and 84 bp, respectively, for the homozygous A/A samples (Figure 1) [15].

Results

A total of consecutive 270 pregnant women with jaundice are enrolled for the study from antenatal wards, antenatal clinic and medical wards of Lok Nayak hospital, New Delhi. Out of 270 cases, 142 showed positivity to HEV infection so included in the study group and age & gestational age matched 142 healthy pregnant women are recruited as pregnant control. The patients with HEV infection are further divided on the basis of severity of disease into AVH group (acute viral hepatitis) or ALF group (Acute liver failure). The AVH group included 103/142 (72.54 %) and ALF group 39 /142 (27.46 %).

When the pregnancy outcome is compared between pregnant women with HEV infection and pregnant control, the preterm delivery is found to be statistically significantly higher 75.6% in pregnant women with HEV infection as compared to 18.3% in pregnant controls. The occurrence of low birth weight babies is also statistically significantly higher in pregnant women with HEV infection (75%) than pregnant controls (25.3%) (p value 0.0001). The maternal and fetal mortality is also found to be statistically significantly higher in

pregnant women with HEV infection than pregnant controls (p value <0.0001) (Table 1).

Table 1: Comparison of adverse pregnancy outcomes between different study groups and control group

Adverse Pregnancy Outcomes	Women with HEV infection (N=142)	AVH group (N=103)	ALF group (N=39)	Control group (N=142)	p value		
	1	2	3	4	1vs4	2vs4	3vs4
Preterm delivery (<37 weeks)	98/128	77/103	21/25	26/142	0.0001*	<0.0001*	<0.0001*
Low birth weight (<2500g)	96/128	76/103	20/25	36/142	0.0001*	<0.0001*	<0.0001*
Total fetal mortality †	29/142	6/103	23/39	0/142	<0.0001*	0.07	0.0001*
Maternal mortality#	24/142	0/103	24/39	0/142	<0.0001*	-	<0.0001*

Before delivery =14 & After delivery =10; †fetal death due to maternal mortality=14 & intrauterine fetal death=15; * p value significant

Table 2: Comparison of ESR2 genotypes for AluI restriction site between adverse pregnancy outcomes in different groups

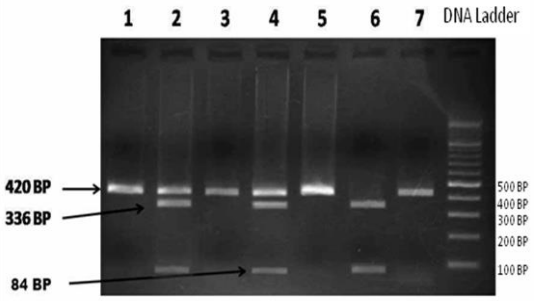
ESR2β genotypes for AluI restriction site	Women with HEV infection (N=142)		AVH group (N=103)		ALF group (N=39)		Control group (N=142)	
	Preterm delivery N=98	p-value	Preterm delivery N=77	p-value	Preterm delivery N=21	p-value	Preterm delivery N=26	p-value
GG	60		53		7		14	
GA	27	0.08	16	0.44	11	0.05	11	0.54
AA	11		8		3		1	
AA	11	0.21	8	0.44	3	1.00	1	1.00
GA+GG	87		69		18		25	
G allele	147	0.01*	122	0.15	25	0.04*	39	0.45
A allele	49		32		17		13	
	Low birth weight (N=96/128)		Low birth weight (N=76/103)		Low birth weight (N=20/25)		Low birth weight (N=36/142)	
GG	57		50		7		23	
GA	27	0.009*	17	0.05	10	0.18	12	0.36
AA	12		9		3		1	
AA	12	0.03*	9	0.11	3	1.00	1	1.00
GA+GG	84		67		17		35	
G allele	141	0.0004*	117	0.004*	24	0.13	58	0.87
A allele	51		35		16		14	

Further, comparison is done for pregnancy outcome between AVH group, ALF group and pregnant controls. It is found that the occurrence of preterm delivery and low birth weight babies are significantly higher in AVH group (77.8% & 73.8% respectively) and ALF group (84% & 80% respectively) compared to pregnant controls (18.3% & 25.3% respectively) (p value <0.0001). The maternal and fetal deaths are found to be statistically significantly higher in ALF group (61.5% & 23.1%) than AVH group (0% & 5.8% respectively) (p value <0.0001) (Table 1).

The comparison of adverse pregnancy outcomes with ESR-beta gene polymorphism for AluI restriction site revealed the occurrence of variant A allele to be statistically significantly higher in preterm delivery (25%) than full term (10%) delivery and in mothers of low birth weight babies (26.6%) than average birth weight babies (6.3%) in pregnant women with HEV infection (p value 0.01 & 0.0004 respectively). The occurrence of variant A allele is statistically significantly higher in preterm delivery (40.5%) than full term (0%) delivery in ALF group (p value 0.04), and in the mother of low birth babies (23%) than average birth weight babies (5.5%) in AVH group (p value 0.004) (Table 2).

The other adverse outcomes like small for date babies, maternal and fetal deaths did not show any association with ESR-beta gene polymorphism for Alu I restriction site.

Figure 1: Representative electrophorosis gel photograph showing ESR beta genotypes for restriction site AluI.



Lane: 1,3,5 & 7 Homozygous GG, Lane: 2 & 4 Heterozygous GA Lane 6 : Homozygous AA, Lane 8: 1 kb DNA ladder

Discussion

In the present study occurrence of preterm delivery, low birth weight babies, maternal and fetal mortality are statistically significantly higher in pregnant women with HEV infection than pregnant control (p value 0.0001). Further, the occurrence of preterm delivery and low birth weight babies are significantly higher in AVH group and ALF group compared to pregnant controls (p value 0.0001). Maternal and fetal deaths are statistically significantly higher in ALF group than AVH group. Similar findings have been reported by studies from India [1, 3-5], though few studies showed no such association [16, 17].

The reason lying behind such fatal outcome is being studied from long time. Jilani et al., (2007) found that HEV-infected pregnant women with FHF had lower CD4 counts and higher CD8 counts and higher levels of estrogen, progesterone and β-HCG in HEV-infected pregnant women with FHF compared to HEV-negative patients or control healthy pregnant females [7]. Bose PD et al., 2011 proposed that PROGINS carriers and lower expression of PR and PIBF, as well as high viral load influences Hepatitis E disease severity and outcomes in pregnancy [8].

Estrogen plays a vital role in viral replication and higher morbidity in pregnant women infected with HEV. In the present study the gene polymorphism of estrogen receptor-beta for Alu I restriction site is studied. The association of ESR-beta gene polymorphism with adverse pregnancy outcome is reported for the first time. The occurrence of variant A allele for AluI restriction site is significantly higher in mothers with HEV infection who had preterm delivery and had low birth weight babies proposing it to be a risk factor for preterm delivery OR 2.989 (95% CI = 1.265-8.084, $p<0.05$) and low birth weight babies OR 5.399 (95% CI= 2.01-18.26, $p<0.05$) in pregnant women with HEV infection. A study reported the frequency of the allele A of Alu I polymorphism in exon 8 to be significantly higher in ICP group than in control group (P=0.031, OR=1.975) and similarly another study also reported higher frequencies of A allele respectively, 21.43% and 10.95% in the ICP patient than control groups OR 2.2174 (95% CI = 1.2866-3.8215, $P<0.05$) [18, 19]. These studies proposed ERβ gene polymorphisms to be associated with ICP and A allele of ESR-beta gene polymorphism for AluI restriction site to be a risk factor for ICP.

Further, the occurrence of variant A allele of ESR beta for AluI restriction site is significantly higher in mother of low birth babies in AVH group with OR 5.056 (95% CI= 1.634-21.57, $p<0.05$) and preterm delivery in ALF group (p= 0.04). A studied reported the variant A allele of ESR beta for Alu I restriction site to be associated with an increased HCV infection susceptibility in the males (additive model: adjusted OR=1.493, P=0.010) and in females carrying AA genotype appeared to clear HCV spontaneously more readily (adjusted OR=0.237, P=0.011).

Conclusion

The higher occurrence of variant A allele for AluI restriction site of ESR-beta gene polymorphism is found to be associated with preterm delivery and low birth weight in pregnant women with HEV infection, preterm delivery in ALF group and low birth weight babies in AVH group. However, severity of disease along with immunological, viral and other hormonal factors may be responsible for adverse pregnancy outcome in HEV infection.

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