List of 10 Best Papers of Dr P. Kar

S.NO	PUBLICATIONS	IMPACT FACTOR	CITATION
1.)	Bose PD, Das BC, Hazam RK, Kumar A, Medhi S, Kar P. **Evidence of extrahepatic replication of hepatitis E virus in human placenta.** Journal of General Virology 2014 Jun 1:95(6):1266-71. **Abstract** The incidence and severity of hepatitis E virus (HEV) infection in pregnant women is high in developing countries. Transplacental transmission of HEV in the third trimester of pregnancy has been found to be associated with high fatal mortality. Based on this evidence and in the absence of reports on HEV replication in extra hepatic sites, this study was carried out to investigate if HEV replication occurs in the placenta of infected mothers. The study included 68 acute viral hepatitis (AVH) and 22 acute liver failure (ALF) pregnant patients. Viral RNA was extracted from blood and placenta. HEV replication in placenta was confirmed by a replicative negative-strand-specific reverse transcriptase PCR. Viral load was estimated by real-time PCR. Immunohistochemical studies were also carried out for in situ detection of HEV in placental tissue sections. Replicative HEV RNA was detectable only in the placenta in ALF and AVH cases and not in blood samples. Positive staining of placental tissue sections with HEV antibody against the viral structural protein ORF3 was observed. HEV replication in placenta also correlated with fatal and maternal mortality in ALF patients. It is demonstrated for the first time that HEV replication occurs in human placenta and that placenta may be a site of extra hepatic replication of HEV in humans.	3.36	112

2.)	Bose PD, Das BC, Kumar A, Gondal R, Kumar D, Kar P. **High viral load and deregulation of the progesterone receptor signalling pathway: association with hepatitis E-related poor pregnancy outcome.** Journal of hepatology 2011 Jun 1:54(6):1107-13	**30.08**	**149**

Abstract

Background & Aims: Hepatitis E virus (HEV) infection is associated with high maternal and fetal mortalities. A prospective study was undertaken to evaluate the role of viral and host factors in HEV related pregnancy outcomes.

Methods: The study included HEV infected pregnancy cases; acute viral hepatitis (AVH), n=100 and fulminant hepatic failure (FHF), n=43, and healthy pregnancy cases, n=50. HEV genotypes and viremia were studied by nucleotide sequencing and real time PCR, respectively. Progesterone receptor (PR) gene mutations (PROGINS) were studied by PCR, PR expression at the mRNA and protein levels in the placenta were studied by semi-quantitative RT-PCR and immunohistochemistry, respectively. Progesterone induced blocking factor (PIBF) expression was studied by RT-PCR in blood. Serum interleukin-10 (IL-10) and interleukin-12 (IL-12) levels were assayed by ELISA.

Results: HEV viral load was significantly higher in FHF than AVH ($p<0.001$) and in cases with fetal mortality in AVH ($p=0.001$) and FHF ($p=0.018$). PROGINS were 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 expression was lower in AVH and even lower in FHF compared to healthy controls. The higher IL-12/IL-10 ratio observed in FHF compared to other groups correlated with fetal mortality in AVH and FHF ($p<0.001$).

Conclusions: In conclusion, reduced expression of PR and PIBF, a higher IL-12/IL-10 ratio, and a high viral load results in poor pregnancy outcome in Hepatitis E.

3.)	Kar P, Jilani N, Husain SA, Pasha ST, Anand R, Rai A, Das BC. **Does hepatitis E viral load and genotypes influence the final outcome of acute liver failure during pregnancy?** Official journal of the American College of Gastroenterology) ACG. 2008 Oct 1.103(10):2495-501.	22.68	148

Abstract

BACKGROUND - Hepatitis E is a major health problem in developing countries including India. The incidence and mortality rate in pregnant women with fulminant hepatic failure (FHF) due to hepatitis E virus (HEV) has been reported to be significantly higher, specifically in Asian women. Pregnancy is usually associated with an altered status of sex steroid hormones and immunity. Steroid hormones directly influence the replication through their effects on viral regulatory elements. Moreover, pregnant women in Asia generally suffer from folate deficiency, which is known to cause reduced immunocompetence leading to greater risk of multiple viral infections and higher viral load.

OBJECTIVES - To correlate and analyze the viral load and genotypes of HEV in acute liver failure with that of acute viral hepatitis among pregnant and nonpregnant women.

MATERIALS AND METHODS -A total of 100 FHF and 150 acute viral hepatitis (AVH) patients (50, 75 pregnant and 50, 75 nonpregnant, respectively), were included in the study. These cases were evaluated on the basis of history, clinical examination, liver function profile, and serological test of hepatitis A, B, C, and E using commercially available ELISA kits. Quantification of HEV RNA-positive samples was carried out.

RESULTS - Out of 100 FHF and 150 acute viral hepatitis (AVH) patients, 28 (56%) and 22 (29.3%) pregnant and 7 (14%) and 8 (16%) nonpregnant, respectively, were HEV RNA-positive. HEV viral load in FHF pregnant women was $5.87 \times 10^4 \pm 1.5 \times 10^5$ μL/mL as compared to AVH pregnant women 343.29 ± 216.44 μL/mL and FHF and AVH nonpregnant 199.2 ± 225.5 μL/mL and 13.83 ± 7.8 μL/mL, respectively. Sequencing data of all the positive samples of FHF and AVH pregnant and nonpregnant women showed genotype 1.

CONCLUSION - HEV viral load was found to be significantly higher ($P < 0.05$) in pregnant patients compared to the nonpregnant. Pregnancy appears to be a risk factor for viral replication. The viral copies of HEV in FHF pregnant women were comparatively higher when compared to AVH pregnant women, which may be related to the severity of the disease in these patients. We could detect only one genotype (genotype 1) in our study population. Thus in the absence of other genotypes in this population, the impact of genotype could not be adequately assessed in this study.

4.)	Singh S, Daga MK, Kumar A, Husain SA, Kar P. **Role of oestrogen and its receptors in HEV-associated feto-maternal outcomes.** Liver International, 2019 Apr; 39(4):633-9.	**5.82**	**13**

Abstract

Background - Pregnant women infected with HEV develops adverse pregnancy outcomes like, abortions, intrauterine fetal death, still births, neonatal deaths, preterm delivery and maternal mortality.

Aim -To correlate oestrogen and its receptors ESR1α and ESR2β levels with HEV-associated feto-maternal outcomes.

Material & Methods - A total of 142 pregnant women with HEV infection and 142 pregnant controls were included in study from Department of Obstetrics & Gynaecology and Department of Medicine, Maulana Azad Medical College (MAMC) and associated Lok Nayak Hospital (LNH), New Delhi. Three millilitre of blood sample was collected in plain for quantification of oestrogen, and its receptors ESR1α and ESR2β using commercially available third-generation ELISA kits.

Results -The levels of oestrogen, ESR1α and ESR2β were considerably higher in HEV-infected pregnant women (20.11 ± 18.19 ng/mL, 10.58 ± 3.27 ng/mL, 10.42 ± 4.71 ng/mL respectively) than pregnant controls (11.74 ± 6.42 ng/mL, 9.11 ± 1.63 ng/mL, 9.01 ± 1.18 ng/mL respectively)($P < 0.0001$). It was found that oestrogen levels were significantly higher in pregnant women infected with HEV who had preterm delivery, low birth weight babies and fetal loss (19.64 ± 17.60 ng/mL, 19.71 ± 17.63 ng/mL, 33.62 ± 23.20 ng/mL respectively) than who had full term delivery, average birth weight babies and live babies (11.71 ± 8.77 ng/mL, 11.99 ± 9.44 ng/mL, 16.58 ± 14.98 ng/mL respectively)($P < 0.05$). A significant negative correlation was observed between baby birth weight and oestrogen levels in HEV-infected pregnant women.

Conclusion -The high level of oestrogen plays an important role in preterm delivery, low birth weight babies and fetal mortality in pregnant women with HEV infection through placental dysfunction. Moreover, oestrogen level is a significant predictor for preterm delivery and maternal mortality and ESR2β levels is a significant predictor for maternal mortality in pregnant women infected with HEV.

5.)	Borkakoti J. Ahmed G, Hussain SA, Rai A, Kar P. **Novel molecular alterations in the ORF 2 capsid gene of hepatitis E virus in patients with acute liver failure in North India.** Archives of virology. 2014 Dec; 159(12):3391-4. **Abstract** Hepatitis E virus (HEV) is evolving as a major global threat to public health, including in developed countries. We partially sequenced the ORF 2 capsid protein genes of HEV genomes from patients with acute liver failure, including pregnant women in the northern part of India. Five unique synonymous substitutions and one non-synonymous substitution, along with a novel mutation, P259S, in the capsid gene, were identified that might be associated with the poor outcome in the patients. Phylogenetic analysis revealed that the isolates belonged to genotype 1 with subtype 1a. The significance of these findings for disease pathogenicity needs to be investigated further.	2.57	11

6.)	Devi SG, Kumar A, Kar P, Husain SA, Sharma S. **Association of pregnancy outcome with cytokine gene polymorphisms in HEV infection during pregnancy.** Journal of Medical Virology. 2014 Aug:86(8):1366-76 Abstract Hepatitis E virus (HEV) infection is associated with high maternal and fetal mortalities. The aim of the study was to find cytokine gene polymorphisms in relation to HEV infection during pregnancy. A total of 262 pregnant and 208 non-pregnant women with hepatitis, 262 healthy pregnant and 208 non-pregnant women as controls. The study group were pregnant and non-pregnant women with HEV infection, not infected with HEV and controls. Genotyping was carried out by PCR-RFLP and ARMS-PCR methods. The frequencies of TNF-α −308 A allele & AA genotype, IFN-γ +874 T allele & TT genotypes were significantly higher in pregnant women with HEV infection compared to other groups. The frequency of TGF-β1 codon 10 +869 T allele &TT genotype and codon 25 +915 G allele & GG genotype were significantly higher in pregnant women compared to non-pregnant women with HEV infection. The frequency of IL-6-174 GG genotype was significantly higher in pregnant women with HEV infection compared to not infected with HEV and controls. Cytokine gene polymorphisms shows association with preterm delivery (TNF-α −308 AA, IFN-γ +874 AA, TGF-β1 codon 10 +869 TT & codon 25 GG genotypes), low birth weight (TNF-α −308 GG & IL-6 −174 CC genotypes), fetal loss (IL-6-174 CC genotype), and small for date (IL-6-174 CC & TGF-β1 codon 10 +869 TC genotypes) of HEV infected pregnant women compared to not infected with HEV and controls. These findings suggest that cytokines gene polymorphisms were found to be associated with pregnant women with HEV infection and adverse pregnancy outcome. ***J. Med. Virol. 86:1366–1376, 2014.*** © 2014 Wiley Periodicals, Inc.	**20.69**	**24**

7.)	Mishra S, Borkakoti J, Kumar S, Kar P. **Role of HEV antigen detection in HEV-related acute viral hepatitis and acute liver failure**. Journal of Medical Virology. 2016 Dec; 88(12):2179-85.	20.69	10

Abstract -

Detection of HEV antigen presents as an interesting low cost, novel, and rapid diagnostic technique to ascertain HEV viremia where facilities for reverse transcriptase polymerase chain reaction (RT PCR) are sparse. This study was undertaken to assess the relative efficacy of HEV antigen detection by ELISA with currently available diagnostic tests in patients of HEV-related acute viral hepatitis (AVH) and acute liver failure (ALF). This study included 36 ALF and 64 AVH cases. HEV RNA and HEV viral load were determined by RT PCR and real time PCR, respectively. Evidence of recent HEV infection was detected in 45/64 AVH cases and 22/36 ALF cases. IgM anti-HEV antibody, HEV RNA, and HEV antigen were positive in 34/45 (75.56%), 26/45 (57.77%), and 21/45 (46.66%), in the AVH group, and 16/22 (72.72%), 14/22 (63.63%), 12/22 (54.54%) in ALF group, respectively. The concordance between HEV RNA and HEV antigen was 75.56% ($P < 0.01$) with κ-coefficient of 0.516 and 75.27% ($P = 0.07$) with κ-coefficient of 0.441 ($P = 0.07$) in the AVH and ALF patients, respectively, indicating moderate concordance. It was established that HEV antigen detection can be used as a valuable marker of active viremia and a cheaper surrogate to HEV RT PCR, particularly in window period, pregnant and immunocompromised patients, however, it did not correlate with severity of disease or influence the final outcome of illness in any of the study groups. ***J. Med. Virol 88:2179–2185, 2016.***

8.)	Borkakoti J, Ahmed G, Ral A, Kar P. **Report of novel H105R, D29N, V27A mutations in the methyltransferase region of the HEV genome in patients with acute liver failure.** Journal of Clinical Virology. 2017 Jun 1:91:1-4. **Abstract -** **Background -** The Hepatitis E virus (HEV) has been responsible for major outbreaks in the developing countries affecting millions of people and acute sporadic hepatitis worldwide. The HEV methyltransferase is important for capping the 5'-end of the viral pregenomic RNA which is critical for viral infection. **Objectives -** We aimed to assess the substitutional profile in the HEV methyltransferase region in patients with acute liver failure (ALF) and acute viral hepatitis (AVH) from North Indian population and associate the substitutions with the poor outcome of the disease. **Study design -** HEV RNA was detected and partial region encoding the Methyltransferase domain in the HEV genome was amplified by Reverse Transcriptase(RT-PCR). Viral load of HEV was quantified utilizing Real time PCR.32 representative samples consisting of 16 AVH and 16 ALF were directly sequenced and amino acid changes were compared using Fischer's exact (two-tailed) test. **Results -** Novel mutations Valine27Alanine (V27A), Aspartate29Asparagine (D29N) and Histidine105Arginine (H105R) mutation corresponding to 107T > C, 115G > A and 341 A > G substitutions respectively were significantly ($p < 0.0001$) obtained in 16/16(100%) ALF patients compared to none (0/16) of the AVH patients. HEV viral load and disease severity parameters corresponding to the samples with D29N and V27A mutations were significantly higher compared to the isolates lacking these mutations while the H105R mutation was associated with decreased viremia. **Conclusion -** The D29N and V27A mutations had significant association with the poor outcome in ALF patients suggesting key role in enhancing HEV replication while the association of H105R mutation with decreased viremia creates interest on its antiviral aspects.	**14.48**	**10**

9.)	Jilani, Nishat et al. "**Hepatitis E virus infection and fulminant hepatic failure during pregnancy.** Journal of gastroenterology and hepatology vol. 22,5 (2007): 676-82.doi:10.1111/j.1440-1746.2007.04913.x **Abstract -** **Background and aim:** Hepatitis E virus (HEV) infection leading to fulminant hepatic failure (FHF) and high mortality is a common feature in Indian women during the second and third trimesters of pregnancy. An altered status of hormones and immunity are observed during pregnancy but the actual cause of high mortality is still unknown. The present study was carried out to analyze CD3, CD4 and CD8 T cell counts and to assay the level of pregnancy-related hormones such as estrogen, progesterone and beta-HCG in order to discover the role played by these factors. **Methods:** One hundred patients (50 pregnant and 50 non-pregnant women) with FHF and 150 pregnant healthy females without liver disease as controls were recruited for the study. Serological tests for all viral markers using ELISA kits and detection of HEV RNA by reverse transcription-polymerase chain reaction (RT-PCR) were carried out in all cases. CD3, CD4 and CD8 T cell counts were analysed by fluorescence activated cell sorter (FACS) while hormone assay was performed by commercially available RIA kits. **Results:** Serologically (38/50; 76%) as well as by RT-PCR (28/50; 56%), a significantly higher HEV positivity rate was found in pregnant FHF patients compared to non-pregnant women (serologically 15/50; 30%; RT-PCR 7/50; 14%). CD4 counts were lower ($P < 0.05$), while CD8 counts were higher ($P < 0.05$), and their ratio (CD4/CD8) in HEV positive pregnant FHF patients was significantly lower ($P < 0.01$) when compared to that of HEV negative pregnant FHF women or controls. Levels of estrogen, progesterone and beta-HCG were also found to be higher ($P < 0.001$) in HEV positive pregnant FHF patients when compared to HEV negative patients or controls. HEV infected pregnant FHF patients had a significantly higher mortality rate of 65.8% (25/38) compared to 23.5% (4/15) in HEV positive non-pregnant women ($P < 0.001$). **Conclusions:** Pregnancy appears to be a potential risk factor for viral replication and an extreme low immune status of Indian/Asian pregnant women. It is suggested that diminished cellular immunity (indicated by a decrease in CD4, an increase in CD8 cell counts and lowered CD4/CD8 cell ratio) and a high level of steroid hormones that influence viral replication/expression during pregnancy appear to be the plausible reasons for severity of the disease.	3.45	260

10.)	Prusty BK, Hedau S, Singh A, Kar P, Das BC. **Selective suppression of NF-kBp65 in hepatitis virus-infected pregnant women manifesting severe liver damage and high mortality.** Molecular medicine. 2007 Sep: 13(9):518-26.	6.35	72

Abstract

Fulminant hepatitis in Asian pregnant women is generally caused by hepatitis E virus infection, and extremely high mortality is most common in them. Decreased cell-mediated immunity is considered a major cause of death in these cases, but what exactly influences decreased immunity and high mortality specifically during pregnancy is not known. We used electrophoretic mobility shift assays, immunoblotting, and immunohistochemical analysis to study the expression and DNA binding activity of NF-kB p50 and NF-kB p65 in pregnant fulminant hepatic failure (FHF) patients and compared them with their nonpregnant counterparts. In both PBMC and postmortem liver biopsy specimens the DNA-binding activity of NF-kB was very high in samples from pregnant FHF patients compared with those from nonpregnant women as well as pregnant women with acute viral hepatitis (AVH) without FHF. Further dissection of the NF-kB complex in supershift assays demonstrated complete absence of p65 in the NF-kB complex, which is formed by homodimerization of the p50 component in pregnant FHF patients. Western blotting and immunohistochemical analysis of the expression of p50 and p65 proteins both showed higher levels of p50 expression and a complete absence or a minimal expression of p65, indicating its nonparticipation in NF-kB-dependent transactivation in pregnant FHF patients. We suggest that the exclusion of p65 from the NF-kB transactivation complex seems to be a crucial step that may cause deregulated immunity and severe liver damage, leading to the death of the patient. Our findings provide a molecular basis, for developing novel therapeutic approaches.

Introduction

Viral hepatitis constitutes a major public health problem in developing countries, including India. In addition to parenterally transmitted hepatitis B and C viruses, which cause majority of hepatitis, enterically transmitted hepatitis E virus (HEV) infection is mainly responsible for sporadic as well as large waterborne hepatitis epidemics related to poor hygiene and sanitation. HEV-induced viral hepatitis is the most common cause of death in Indian pregnant women (1). Studies carried out in Iran, Africa, the Middle East, and other Asian countries have also found a high mortality due to fulminant hepatic failure (FHF) during pregnancy in women with HEV infection (2–5). In contrast, reports from the United States and Europe have failed to find any significant correlation between death during pregnancy and viral hepatitis (6). The mortality rate in pregnant women with FHF has been found to be specifically higher during second and third trimesters of pregnancy (1,2,7–9), which are associated with an altered status of hormones and immunity, but what exactly influences high mortality during pregnancy is not known.

NF-kB, a eukaryotic dimeric transcription factor formed by hetero- or homodimerization of proteins of the Rel family, is involved in a wide range of cellular effects, including immune and inflammatory responses, proliferation, cell survival, and apoptotic stimuli (10). Different members of the Rel family, such as p50, p52, p65, cRel, and RelB possess a *rel* homology domain that confers DNA

binding and protein dimerization properties (11). NF-kB remains in an inactive form in the cytoplasm by binding to the labile cytoplasmic inhibitor IκB (12,13), which masks the RelA nuclear localization signal. The release of IκB in response to intracellular signals leads to nuclear translocation of the p50 and p65 subunits and subsequent activation of a whole set of NF-kB responsive effector genes. Disruption of the RelA locus in mice lacking the p65 subunit of NF-kB has been demonstrated to lead to embryonic lethality at 15–16 d of gestation due to massive degeneration of the fetal liver by programmed cell death (14). Studies done with p65 knock-out mice also indicated that p65 is indispensable for liver development and causes enhanced cell proliferation during embryonic development (9). This finding prompted us to investigate the probable role of NF-kB during the death of hepatitis virus infected pregnant women with FHF. We report that the suppression of p65 expression appears to be associated with the breakdown of immunity and with severe liver degeneration leading to death of the patient.