Abstract
Abstract:

Background & Aims:

Hepatitis E, a major public health concern in developing countries, is responsible for sporadic and epidemic acute viral hepatitis in adults. Hepatitis E virus (HEV) is predominantly transmitted via fecal-oral route, mainly through contaminated water. During epidemics, HEV is known to be responsible for high mortality among pregnant women (Antenatal care, ANC) especially in the third trimester. In the sporadic setting, men and non-pregnant women have been shown to progress / succumb to fulminant hepatic failure. In the absence of a convenient laboratory animal model or a robust cell-culture system, the progress in the understanding of the pathogenesis of hepatitis E has been rather slow. Though primates have been shown to be susceptible to HEV, infection of rhesus monkeys in the third para of pregnancy did not develop severe disease. Overall, pathogenesis of hepatitis E, especially during pregnancy, is poorly understood. Considering the added importance of innate immunity during pregnancy, our aim was to assess the role of Toll-like-receptors (TLRs), monocytes and T cells in self-limiting hepatitis E with (ANC) or without pregnancy (non-ANC).

Study subjects and Methods:

The patient categories included non-ANC-patients during the acute (n=46), early-convalescent (n=32) and convalescent (n=31) phases of the disease and ANC patients (2nd and 3rd trimesters, n=13). The controls and subclinical HEV infections respectively included: non-ANC (n=30 and 5), ANCs in the first (n=10 each) and later (n=10 and 20) trimesters. Flow cytometry was used to determine the levels of TLR2, TLR3, TLR4, TLR7, TLR8 and IRAK4, IkBα, pNFkB, TBK1, IRF7. For monocytes and T cells, whole blood was stained with CD4, CD14, CD28, CD80, CD86, CD137, CD152 and HLA-DR antibodies. Cytokine responses induced by TLR-specific ligands-stimulated PBMCs from ANC and non-ANC patients and TLR signaling molecules (non-ANC patients) and plasma levels of IL6, IL8, IL12 and TNFα were measured by milliplex cytokine kit. PBMCs were used for gene expression analysis by Taqman Low Density Array.
Results:

The levels of TLR 4, TLR7 and TLR8 were significantly higher (p<0.05) during acute phase of disease as compared with anti-HEV negative healthy controls, while TLR2 and TLR3 exhibited comparable levels (p>0.1). At gene levels TLR3, TLR4, TLR7 and TLR8 were higher during acute phase of disease. When ANC subjects with or without HEV infection were compared, TLR2, TLR3, TLR4, TLR7 and TLR8 levels were comparable (p>0.1), whereas Acute-ANC (2nd+3rd trimesters) patients exhibited significantly lower levels of TLR2, TLR3, TLR4, TLR7 and TLR8 when compared to acute-non-ANC (p<0.01). However, the ANC-controls (2nd+3rd trimesters) exhibited lower levels of TLR2, TLR3, TLR4 and TLR8 when compared to the non-ANC controls (p<0.05) while in the first trimester, the levels of TLR2, TLR3 and TLR4 were lower (p<0.01). When gene levels of TLRs were compared none of TLR was changed during pregnancy, indicative of impaired TLR response.

Stimulation of PBMCs from the respective groups with TLR-specific ligands led to the induction of type-I interferons, IFNβ by the non-ANC group and IFNα by the ANC category. During the acute phase, higher levels of TBK1, IRF7 (p<0.01, TLR3-ligand), TBK1, IRF7, NFκB (p65), IRAK4, IκBα (p<0.05, TLR4-ligand) and IRF7 (p<0.05, TLR7/8-ligand) were observed. In contrast to the absence in the ANC-patients, involvement of MyD88-independent (TLR3), MyD88-dependent / independent (TLR4) and MyD88-dependent (TLR7 and TLR8) pathways was shown in the non-ANC-patients.

We examined two important immune cell types i.e., CD14+ monocytes and CD4+ T cells and respective subpopulations in self-recovering hepatitis E patients with or without pregnancy. The frequency of CD14+, CD14+CD80+, CD14+CD86+, CD14+HLA-DR+ monocytes was significantly higher in the non-ANC-patients than in the controls (p<0.001), whereas, CD14+CD209+ cells were comparable (p>0.1). The healthy pregnancy was associated with increased frequency of CD14+: CD14+CD80+, CD14+CD209 monocytes and diminished expression of CD14+HLA-DR+ (p<0.05) when compared to the non-ANC controls. When ANC-patients were compared with non-ANC patients, elevated levels of CD14+ (p<0.01) and CD14+CD80+ monocytes (p<0.001) were observed in the ANC patients while reduction in the CD14+HLA-DR+ and CD14+CD86+ monocytes (p<0.05) were observed in the
ANC patients. The frequency of TLR2$^+$ and TLR4$^+$ monocytes was higher in the non-ANC-patients (p<0.00) as compared to the non-ANC controls and lower in the ANC-patients (p<0.01) than the non-ANC patients. The control ANC$^+$s exhibited lower expression of CD14$^+$TLR4$^+$ cells (p<0.05) as compared to the non-ANC controls. Comparable levels of monocytes and its subpopulations were observed in the ANC patients when compared to the ANC controls.

Compared to the respective controls, CD4$^+$CD137$^+$, CD4$^+$CD152$^+$ and CD4$^+$CD278$^+$ cells were higher (p<0.05) in both patient categories, while the levels of CD4$^+$,CD4$^+$CD28$^+$ were comparable (p>0.1). Healthy pregnancy was associated with higher levels of CD4$^+$CD152$^+$ and CD4$^+$CD278$^+$ T cells (p<0.001). Among patients groups, the CD4$^+$ or the subpopulations of CD4$^+$T cells were not different (p>0.05). We found higher and reduced levels respectively of circulating plasma cytokines (IL12, TNF$\alpha$, IL6 and IL8) in the non-ANC patients than in the ANC-patients. Healthy pregnancy exhibited higher levels of IL8 and TNF$\alpha$ when compared to the non-ANC controls. However, pregnancy with disease showed lower levels of IL12p70 and TNF$\alpha$ than in the ANC controls.

**Conclusions:**

The study concludes that:

1. TLR4, TLR7 and TLR8 are associated with recovery in the non-ANC-patients.
2. The normal pregnancy among rural Indian women from Maharashtra, India is associated with lower TLR response at both protein / gene levels and pro-inflammatory plasma cytokine response.
3. Higher plasma pro-inflammatory cytokines are associated with recovery in the non-ANC patients.
4. The disease in pregnancy could not alter the diminished TLR response and induces lower pro-inflammatory response.
5. Except for the robust type-I-interferon response, HEV infection could not modulate pregnancy-related diminished immune response.
6. On contrary to the classical activation of CD14$^+$monocytes in the non-ANC-patients, impaired response was evident in the ANC-patients while the CD4$^+$T cell populations were similar in the patient groups.
These results have important implications in the understanding of HEV pathogenesis and form basis for further, most needed, evaluation of the fulminant hepatitis E patients with and without pregnancy and leading either to recovery or death.