Background & Aim: Ammonia plays a central role in the pathogenesis of brain oedema in acute liver failure (ALF) and experimental models have unequivocally associated ammonia exposure with astrocyte swelling. Infection and systemic inflammation are common in ALF, drive the development of intracranial hypertension (ICH) and are major prognosticators. We have recently reported that patients with ALF and brain oedema have increased neutrophil TLR9 expression and that this can be induced synergistically by ammonia and IL-8. We hypothesised that ammonia-induced brain oedema and immune dysfunction are mediated by TLR9. This study aimed to determine whether this could be prevented in a hyperammonemic TLR9 deficient (TLR9-/-) mouse model. Methods: Ammonium...
acetate (NH4-Ac) (4mmol/kg) was injected intraperitoneally in C57Bl/6 mice - wild type (WT), TLR9-/- and TLR9fl/fl LysCre (TLR9 absent in neutrophils and Kupffer cells). Six hours after NH4-Ac injection, mice were sacrificed, organs harvested and changes compared to controls (without NH4-Ac stimulation). Cells from spleen and liver were isolated to determine intracellular cytokine (IFN-γ, TNF-α & IL-6) production of T-cells, macrophages and natural killer cells. Brain and liver were isolated to determine brain water content and liver bodyweight ratio. Total plasma DNA (tDNA) was measured using the Picogreen dsDNA kit. The role of the TLR9 antagonist (ODN2088–50µg) injected simultaneously with NH4-Ac was also evaluated. The effect of a single toxic dose of NH4-Ac (12mmol/kg) was also evaluated. Results: Following NH4-Ac injection, cytokine production was significantly increased (p<0.0001) in WT mice compared to controls. This was accompanied by increased brain water content (Figure-1a) and liver bodyweight ratio (p<0.0001). Following NH4-Ac injection, cytokine production, brain water content and liver bodyweight ratio were significantly decreased (p<0.01) in TLR9-/- mice compared to WT mice. When ODN2088 was administered with NH4-Ac, cytokine production was significantly decreased (p<0.05) and increased brain water content and liver bodyweight ratio (p<0.0001) were prevented (Figure-1b). Following NH4-Ac injection, cytokine production of macrophages and brain water content were significantly decreased in TLR9fl/fl LysCre mice compared to WT mice (p<0.05) indicating that neutrophil TLR9 has an important role. Following NH4-Ac injection, plasma tDNA levels were significantly increased in WT and TLR9-/- mice compared to controls (p<0.05) indicating that TLR9 was activated through the DNA released by the ammonia-stimulated cells. There was reduced mortality in TLR9-/- mice compared to WT mice (p=0.0007) following the toxic dose of NH4-Ac injection (12mmol/kg) indicating that TLR9 plays an important role in ammonia-induced pathophysiological changes and mortality (Figure-1c). Conclusion: In summary, these data show that ammonia-induced brain oedema is mediated through TLR9 in a DNA-dependent fashion. The amelioration of brain oedema and lymphocyte cytokine production by ODN2088 supports exploration of TLR9 antagonism as a therapeutic modality in early ALF to prevent the progression to ICH.

**Figure: The role of TLR9 in ammonia-induced brain oedema and mortality**
(a) Decreased brain water content in TLR9⁻/⁻ mice following NH₄Ac injection (4mmol/kg) compared to WT mice.
(b) Reduced brain water content in WT mice after the administration of ODN2058 along with NH₄Ac (4mmol/kg).
(c) Reduced mortality in TLR9⁻/⁻ mice following a toxic dose of NH₄Ac (12mmol/kg).

**Corresponding Author Detail**

**Name:** Godhev K Manakkat Vijay  **ISHEN Member:** ISHEN Member  **Country:** United Kingdom

**State:** London  **City:** London  **E-mail:** godhevijay@gmail.com

**Mobile:** +447405612213

**Address:** Institute of Liver Studies King's College London School of Medicine at King's College Hospital Denmark Hill

**Do you wish to apply for young investigator bursaries:** yes