Summer 8-12-2021

Role of Liver Stiffness and Alcohol on HBV Infection Pathogenesis

Tabitha Lynn Guyett
Buena Vista University

Grace Bybee

Youra Moeun

Moses New-Aaron
University of Nebraska Medical Center

Srivatsan Kidambi
University of Nebraska - Lincoln

See next page for additional authors

Author

Tabitha Lynn Guyett, Grace Bybee, Youra Moeun, Moses New-Aaron, Srivatsan Kidambi, Natalia Osna, and Murali Ganesan

Role of Liver Stiffness and Alcohol on HBV Infection Pathogenesis

Tabitha Guyett, Grace Bybee, Youra Moeun, Moses New-Aaron, MPH., Srivatsan Kidambi, Ph.D., Natalia Osna, MD, Ph.D., Murali Ganesan, Ph.D.
Research Service, Veterans Affairs Nebraska-Western Iowa Healthcare System, Omaha, NE.
Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE.
Department of Chemical & Biomolecular Engineering, University of Nebraska, Lincoln, NE

Introduction

- Hepatitis B virus (HBV) is an infection that specifically targets the liver cells and is a known cause of liver inflammation and cirrhosis.
- The consumption and metabolism of alcohol is also known to cause liver inflammation and perpetuate HBV infection.
- Ultrasounds have been used to determine the extent of liver damage via measuring the current pressure in a patient's liver tissue.
- Healthy liver tissue has a pressure below 6 kPa. As a liver goes through the stages of fibrosis, this pressure increases. A fibrotic liver has a pressure greater than 12.5 kPa.
- Previous studies have reported that tissue stiffness affects primary hepatocyte function and cell interaction.
- The focus of this study is to investigate the role of liver stiffness in HBV infection and if ethanol metabolism potentiates this relationship.
- Elucidation of the liver environment's role in hepatocyte HBV infection and alcohol metabolism could pave the way to new treatment options for patients as well as introduce more accurate lab models for research.

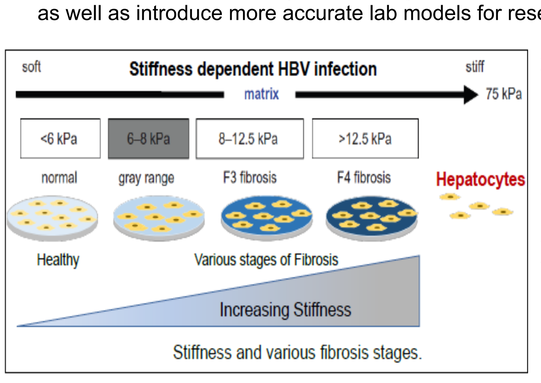


Figure 1. The stages of fibrotic development according to pressure readings. Prior to liver inflammation and fibrosis, a healthy liver will have a pressure lower than 6 kPa. When the liver becomes inflamed and progresses through the stages of fibrosis, the pressure in the liver tissue increases significantly. Once the liver progresses from fibrosis to cirrhosis, liver damage in generally irreversible.

Major Questions

- Does liver stiffness potentiate HBV infection in hepatocytes and if so by which mechanism does it achieve this?
- Does the combination of liver stiffness and ethanol metabolism potentiate HBV infection?

Material and Methods

- **Cells:** HBV transfected HepG2.2.15 cells (HBV Genotype D)
- **Plates:** Plates with a pressure of 2 kPa were used to mimic soft, healthy liver. To mimic a stiff, diseased liver, plates with a pressure of 25 kPa were used.
- **Treatments:** To mimic ethanol metabolism, cells were treated with Acetaldehyde Generating System (AGS): 50 mM ethanol, NAD+, and yeast alcohol dehydrogenase (ADH) for 72 hours
- RT-PCR/ddPCR was used to detect mRNA and DNA levels.
- Western Blot was used to detect protein levels.
- ELISA was used to analyze HBV surface antigen levels in cultured media.

Results

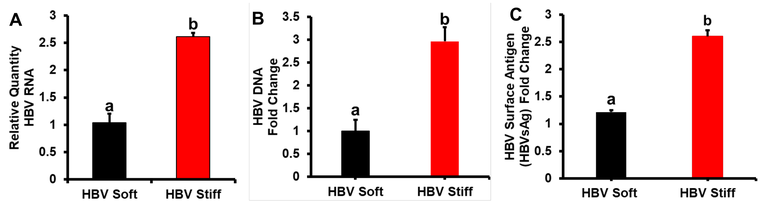


Figure 2. Higher liver stiffness increases HBV infection markers. HBV RNA was measured by RT-PCR (A), HBV DNA was quantified by ddPCR (B), ELISA was used to analyze the HBV Surface Antigen levels (C). Bars with different letters are significantly different with $p \leq 0.05$.

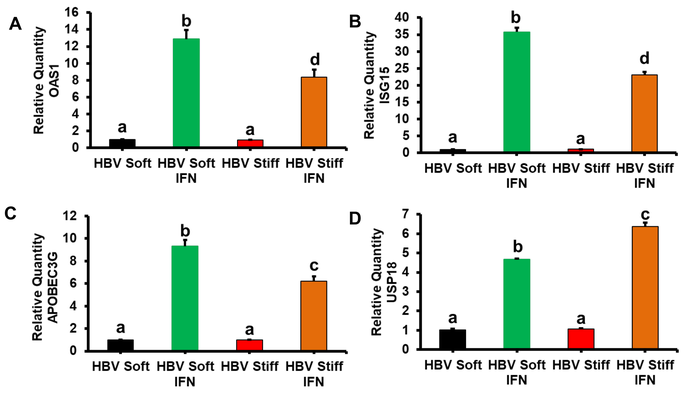


Figure 3. Liver stiffness increases HBV infection by decreasing the innate immune response. mRNA expression of Interferon sensitive genes (ISGs) OAS1(A), ISG15 (B), and APOBEC3G (C) and USP-18 (D) in HepG2.2.15 cells. Bars with different letters are significantly different with $p \leq 0.05$.

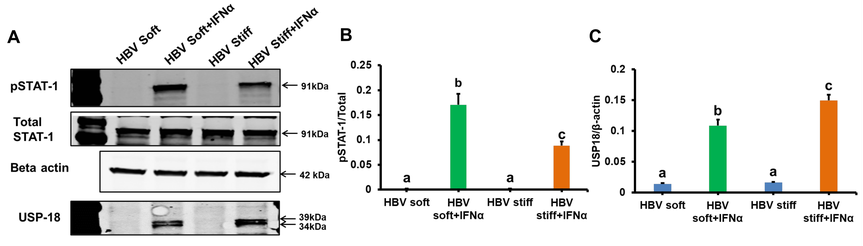


Figure 4. Liver Stiffness impairs the innate immune system via USP-18. Western blot of pSTAT-1, Total STAT-1 and USP-18 and Beta actin as an internal control (A) with quantification (B-C Bars with different letters are significantly different with $p \leq 0.05$.

Acknowledgements

- This project is supported by UNL systems science grant, K01AA0268864 NIH-NIAAA, and the Summer Undergraduate Alcohol Research Program (SUARP) at UNMC.

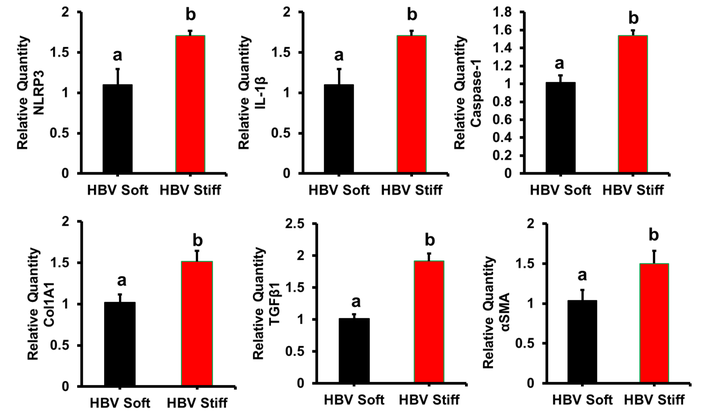
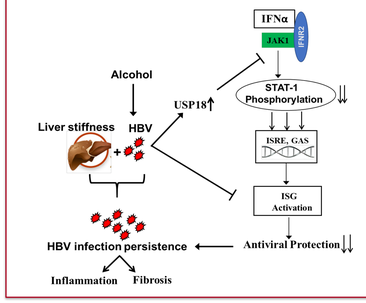


Figure 5. Liver stiffness activates inflammasomes and increases pro-fibrotic markers. mRNA expression of NLRP3 (A), IL1β (B), Casp-1 (C), Col1A1 (D), TGFβ (E), and αSMA (F).

Figure 6. The combination of liver stiffness and alcohol metabolism increase the HBV infection of in HepG2.2.15 cells. HBV RNA (A), DNA (B), and surface antigen levels (C).

Summary

- The presence of HBV infection markers are significantly greater in hepatocytes plated on stiff tissue, which signify a diseased liver (Fig 2).
- The mechanism by which liver stiffness decreases innate immune system response is mediated by the upregulation of USP-18 which down regulates the IFN∝ and JAK/STAT-1 pathway. This leads to an overall decrease in the expression of innate immune response specific genes (Fig 3-4).
- When IFN∝ is added to the HBV soft and stiff model, the stiff model has a significant decrease in pSTAT-1 and a subsequent rise in USP-18(Fig 4).
- Liver stiffness activates both inflammasome and pro-fibrotic markers when compared to the soft liver (Fig 5).
- The combination of liver stiffness and alcohol metabolism, increases HBV infection markers significantly more than stiffness alone (Fig 6).



Conclusion

We conclude that the environment of the hepatocytes, specifically liver stiffness, contributes to potentiation of HBV infection. Those who have a stiff liver and drink alcohol may be at a higher risk of HBV infection. Further understanding of liver stiffness mechanisms will provide new treatment options for patients.