OCCULT HEPATITIS B VIRUS INFECTION (OBI) DETECTION USING AN OPTIMIZED NESTED POLYMERASE CHAIN REACTION (PCR) AND SEQUENCE ANALYSIS OF THE S-GENE OF THE HEPATITIS B VIRUS.

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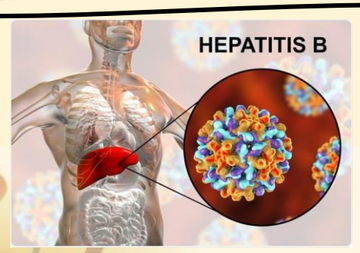
INTRODUCTION

- OBI is a risk factor for chronic liver disease and hepatocellular carcinoma (HCC).
- OBI is defined as HBV DNA detection in serum or in the liver by in HBsAg-negative patients with or without serologic markers of previous viral exposure.
- Naturally occurring mutations happens quite frequently in hepatitis B virus (HBV) due to its replicative strategy.
- These mutants are responsible for false-negative Hepatitis B surface antigen tests.
- The aim of this study is to sequence and identify mutations in the hepatitis B virus surface antigen gene in patients with OBI.



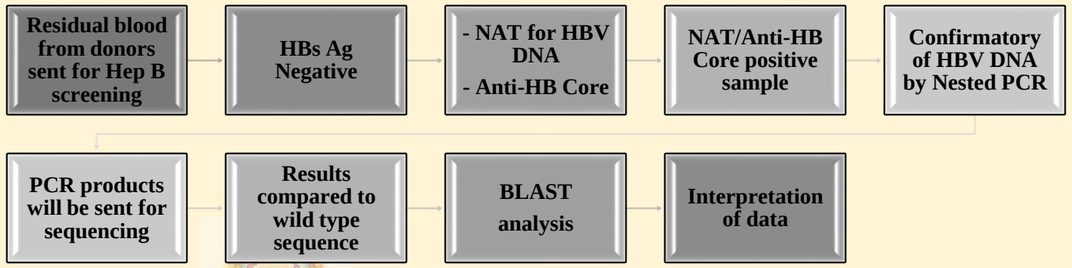
OBJECTIVES

- To determine the proportion of OBI in HBsAg negative hepatitis screening tested using anti-HBc in Hospital USM.
- To optimize the nested-PCR protocol for the detection of OBI from blood samples.
- To do sequence analysis of the S-gene of the hepatitis B virus patients with OBI in Hospital USM.

METHODOLOGY

- This study will be conducted in Hospital Universiti Sains Malaysia, Malaysia.
- Residual blood samples from new and lapsed blood donors are collected and screened using anti-HB core Total serology.
- Positive samples are tested with Nested-PCR which confirms the presence of HBV DNA.
- Primers targeting the HBsAg are used and sera from patients with chronic HBV infection are used as control.
- The PCR products found positive will be sent for sequencing.
- The sequencing results will be compared to wild type sequence obtained from Genbank and analyzed using BLAST software.
- This study will identify the possible genetic cause of OBI.

FLOWCHART



MUTATIONAL ANALYSIS

- Nested PCR is a modification of PCR designed to increase the sensitivity and specificity of the assay reaction.
- It involves the use of two primer sets directed against the same target and two successive PCR reactions.
- Two sets of primers are used to amplify the S gene.
- The first round is targeted for a 916-bp segment of the envelope gene.
- The second round of PCR is performed using the internal primers to amplify 656-bp of the S gene.
- Different sets of primers will be used from similar research papers and used for the amplification.

EXPECTED RESULTS

- The percentage of anti-HBc-positive among HBsAg negative blood tested is reported.
- Positive samples will be confirmed by nested-PCR.
- Sample bands will be compared to the 656-bp band specific for hepatitis B virus *S* gene fragment.

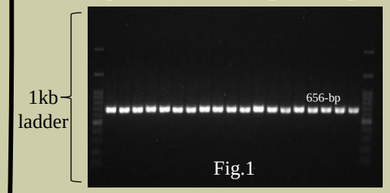


Fig.1 shows the expected electrophoresis gel results.

Source: Naturally occurring hepatitis B virus surface antigen mutant variants in Malaysian blood donors and vaccinees (Hudu et.al)

- Fig.1 shows the expected outcome of the gel results from positive samples.
- The bands are expected to appear around 656-bp of the 1kB ladder.
- Confirmed positive samples will be sent for Sanger sequencing.
- The sequencing results will be compared to wild type sequence obtained from Genbank and analyzed using BLAST software.

KEY REFERENCES

1. Hudu, S. A. et al. (2015) 'Naturally occurring hepatitis B virus surface antigen mutant variants in Malaysian blood donors and vaccinees', European Journal of Clinical Microbiology and Infectious Diseases. Springer Verlag, 34(7), pp. 1349–1359. doi: 10.1007/s10096-015-2358-1.
2. Hudu, S. A. et al. (2016) 'Molecular and serological detection of occult hepatitis B virus among healthy hepatitis B surface antigen-negative blood donors in Malaysia', African Health Sciences. Makerere University, Medical School, 16(3), pp. 677–683. doi: 10.4314/ahs.v16i3.6.

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