PREVALENCE AND GENOTYPE DISTRIBUTION OF HDV VIRUS AMONG CHRONIC HEPATITIS B CARRIERS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

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Fathin Hadi

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LIST OF ABBREVIATIONS

ALT	Alanine Transaminase
AST	Aspartate Transaminase
DAA	Direct antiviral agent
GT	Genotype
HBV	Hepatitis B virus
HDV	HDV virus
Hospital USM	Hospital Universiti Sains Malaysia
RAV	Resistance-associated variant
RNA	Ribonucleic acid
RT PCR	Reverse Transcriptase Polymerase Chain Reaction
cDNA	Complementary DNA / Copy DNA

ABSTRAK (BAHASA MELAYU)

Pengenalan

HDV adalah salah satu jenis virus Hepatitis yang disebabkan oleh Hepatitis Delta Virus. Virus HDV sangat unik di mana, ia memerlukan Hepatitis B untuk pembiakannya. Jangkitan HDV boleh dipamerkan sebagai co-infection (jangkitan Hepatitis B dan D berlaku serentak) atau superinfection (pesakit dijangkiti Hepatitis B dan seterusnya dijangkiti dengan HDV. Kombinasi jangkitan Hepatitis B dan D boleh menyebabkan berlakunya masalah virus hepatits kronik yang bahaya dan boleh menyebabkan kegagalan sel hati yang cepat dan seterusnya menggalakkan pembentukan kanser hati.

Secara global genotip HDV bertaburan mengikut geografi setempat dengan HDV 1 kebiasaannya dijumpai di negara maju seperti Amerika dan di beberapa tempat di negara Eropah. Sementara itu, HDV 3 lebih kerap dijumpai di Selatan Amerika dan bersifat lebih agresif dan boleh menyebabkan Hepatitis fulminan (kegagalan fungsi hati yang cepat). Manakala HDV 2 selalunya dijumpai di negara Asia.

Dengan adanya program immunisasi Hepatitis B secara global, dilihat bilangan jaangkitan HDV di seluruh dunia menurun. Penyakit HDV boleh dicegah dengan mengambil immunisasi untuk hepatits B, namun kadar kejayaan rawatan adalah rendah.

Kaedah

Kajian ini bersifat kajian lintang dan bukan intervensi. Peserta yang memenuhi kriteria positif dan negatif direkrut daripada Hospital Universiti Sains Malaysia bermula pada 1 Februari 2021 sehingga 27 Januari 2023. Parameter klinikal yang terpilih dengan satu set sampel darah akan diambil untuk analisis lebih lanjut. Ekstrak HBV DNA dari serum digunakan untuk menentukan genotip untuk HBV dengan menggunakan primer khusus. Kaedah penjajaran secara terus telah digunakan untuk mengenal pasti Genotip HBV yang terdiri daripada lapan Genotip HBV A hingga G. Kaedah ini dijalankan dengan menggunakan perisian MEGA 5. Pengesanan HDV-RNA adalah melalui protocol RT PCR menggunakan “HDV nested primer” yang khusus bagi mengesan dan menguatkan HDV. Dapatan daripada PCR and seterusnya digunakan untuk mengkelaskan HDV genotip berdasarkan kepada profil RFLP. Sebanyak lapan jujukan prototaip Genotip HDV telah diterima daripada bank Gen NCBI untuk pengaturan and pengenal pastian HDV Genotip.

Keputusan

Sejumlah 226 subjek positif hepatits B telah direkrut dalam kajian ini. Daripada ujian HDV ELISA didapati seramai 26 orang adalah positif HDV. Sebanyak 2 genotip HDV telah dikenalpasti iaitu genotip 1 dengan bilangan 9 subjek (34.6%) dan genotip 2 dengan bilangan 4 subjek (15.4%). Sejumlah 13 subjek (50%) tidak dapat dikenalpasti jenis genotip HDV.

Daripada 26 subjek tersebut, sebanyak 5 jenis sub Hepatitis B telah dikenal pasti; 9 (34.6%) jenis sub B1, 5 (19.2%) jenis sub B2, 7 (26.9%) jenis sub B3, 4 (15.4%) jenis sub B4 and 1 (3.8%) jenis sub B5.

Berdasarkan kajian ini, didapati bahawa tiada perhubungan secara klinikal antara kehadiran sirosis hati dengan genotip HDV tertentu dengan nilai p sebanyak 0.127 ($p>0.05$). Tambahan lagi, kajian ini menunjukkan tiada perhubungan di antara bacaan AST dan ALT dengan genotip HDV dengan nilai p sebanyak 0.821($p>0.05$).

Melalui kajian ini juga, kami tidak dapat membuktikan sebarang perkaitan antara genotip HDV dengan parameter klinikal tertentu.

Kesimpulan

Daripada jumlah 226 subjek, sebanyak 26 subjek didapati positif ujian HDV ELISA. Data yang diperolehi telah menunjukkan bahawa, genotip 1 adalah genotip HDV yang paling lazim ditemui, dengan 9 subjek (34.6%), diikuti oleh genotip 2 dengan 4 subjek (15.4%). Sebanyak 13 subjek (50%) tidak dapat dikenalpasti jujukan genotip disebabkan oleh beberapa factor semasa mengendalikan specimen. Tiada perhubungan yang jelas antara genotip HDV dan manifestasi klinikal ditemui.

Sebanyak 5 sub jenis Hepatitis B telah dikenalpasti dengan genotip Hepatitis B yang paling lazim adalah seperti berikut Hepatitis B Subjenis B1 dengan 9 (34.6%) subjek, Hepatitis B Subjenis B2 dengan 5 (19.2%) subjek, Hepatitis B Subjenis B3 dengan 7 (26.9%) subjek, Hepatitis B Subjenis B4 dengan 4 (15.4%) subjek, and Hepatitis B Subjenis B5 dengan 1 (3.8%) subjek.

ABSTRACT

Background

HDV is one of the viral Hepatitis variants caused by the Hepatitis Delta virus. The HDV virus is unique in that it requires Hepatitis B for its propagation. HDV infection can present as co-infection (both Hepatitis B and D co-occur) or superinfection (subsequent infection with HDV after being infected with Hepatitis B). The combination of both Hepatitis B and D can lead to a severe form of chronic viral hepatitis as it can cause rapid liver cell injury and death, and may lead to the formation of hepatocellular carcinoma.

HDV virus genotypes are known to be geographically distributed globally, with HDV 1 as the most frequently found genotype in high-income countries such as America and among European countries. In contrast, HDV 3, more commonly seen in South America, is more aggressive and may cause fulminant hepatitis. HDV 2 is commonly found in Asia.

Due to the successful global Hepatitis B virus vaccination program, the number of HDV infections is declining worldwide. HDV infection can be prevented by Hepatitis B immunisation, but treatment success rates are low.

Methodology

This study was a non-interventional, cross-sectional cohort study. Participants who fulfilled the inclusion criteria and exclusion criteria were recruited from Hospital Universiti Sains Malaysia from 1st February 2020 till 27th January 2023. Selected

clinical parameters were obtained with one-off blood taking for further analysis. HBV DNA extracted from serum was used for genotyping for HBV by using established specific primers in a two-tail PCR. Direct sequencing methods were used for the HBV genotypes detection, representing the eight HBV genotypes A to G, was performed using the MEGA 5 software. HDV-RNA detection uses RT PCR protocol using a specialised HDV nested primer to detect and amplify HDV. The PCR product will further be used for HDV genotyping according to the RFLP profile. Eight prototype HDV-genotype sequences were retrieved from the NCBI Gene bank for alignment and HDV genotyping.

Results

A total of 226 HBV positive subjects were recruited in the study. 26 subjects were HDV-positive from the HDV ELISA test. A total of 2 HDV genotypes were identified, which were genotype 1 with 9 subjects (34.6%) and genotype 2 with 4 subjects (15.4%). A total 13 subjects (50%) were unable to determine the HDV genotypes.

From these 26 subjects, five subtypes of Hepatitis B were found 9 (34.6%) subtype B1, 5 (19.2%) subtype B2, 7 (26.9%) subtype B3, 4 (15.4%) subtype B4 and 1 (3.8%) subtype B5.

From this study, no clinical association was found between the presence of liver cirrhosis with specific HDV genotypes with a p-value of 0.127 (p-value >0.05). In addition, this study found no association between AST and ALT levels with specific HDV genotypes with a p value of 0.821 (p-value >0.05). From our research, we could not demonstrate any significant association between HDV genotypes and specific selected clinical parameters.

Conclusion

From 226 total subjects, 26 subjects were positive HDV ELISA test. A total of 5 Hepatitis B subtypes were found with the most prevalent genotype of Hepatitis B; Hepatitis B Subtype B1 with 9 (34.6%) subjects, Hepatitis B Subtype B2 with 5 (19.2%) subjects, Hepatitis B Subtype B3 with 7 (26.9%) subjects, Hepatitis B Subtype B4 with 4 (15.4%) subjects, and Hepatitis B Subtype B5 with 1 (3.8%) subject.

The data showed that genotype 1 is the most prevalent HDV genotype, with 9 subjects (34.6%), followed by genotype 2 with 4 (15.4%). We could not identify the HDV genotype sequences for 13 subjects (50%) due to several factors while handling the specimens like the small concentration of the synthesized cDNA from the RNA extraction, the volatility of RNA, and the unstable nature of RNA, the later usually cause loss of RNA integrity causes cDNA to denature early and in some cases produce non readable amplicons during its amplification using nested PCR. There was no significant association found between the HDV genotypes with the clinical manifestation.

CHAPTER 1: INTRODUCTION

Introduction

HDV is caused by the HDV virus (HDV). It is a unique RNA pathogen which needs Hepatitis B virus (HBV) envelope proteins to infect hepatocytes. Subjects with concomitant HBV-HDV infection are at risk of more severe hepatic decompensation during flares. In addition, the co-infection may accelerate liver disease in the long run (1–4). Among 250 million chronic HBsAg carriers worldwide (5), approximately 12.5 million individuals were infected with HDV (6).

HDV is a small single-stranded negative-sense circular RNA virus encompassing a spherical HDAg and HBsAg-hybrid particle of ~36 nm in diameter and an outer envelope containing Hepatitis B surface antigens (HBsAg) (7, 8). The HDV genome consists of 1680 nucleotides which are then covered by 200 HDV antigen molecules (2, 9). HDV requires HBV envelope proteins for assembly, propagation, and transmission to initiate a new infection cycle (6, 10, 11). HDV can be acquired either as a co-infection with acute Hepatitis B or as a superinfection, especially in chronically HBV-infected individuals (11-13).

The HDV virus has been separated into at least eight significant clades based on their genome diversity, with specific geographic distribution (6, 14). HDV-1 is the frequently occurring clade distributed among Europe, the Middle East, America and North Africa (15-18). HDV-2 to HDV-8 occur regionally (19). For instance, HDV-2 prevails in countries like Japan (20), Taiwan (21, 22) and Russia (23). HDV-3, a most diverged genotype, is exclusively found in South America (18, 24, 25). HDV-4 is reported across Japan and Taiwan (21), whereas HDV-5 to HDV-8 are described in Africa (26,

27). The distribution of HDV varies with different geographical regions, with a higher incidence in the Middle East, Mediterranean, Amazonas, Africa, and Asian countries (6, 14, 28-30).

As mentioned above, HDV-1 is ubiquitous, but HDV-3, seen more in South America, is more aggressive and is responsible for outbreaks of fulminant Hepatitis. This underlines that HDV infection, whether in the form of a co-infection or a superinfection, is a significant cause of fulminant viral Hepatitis.

In Malaysia, HDV was first described in 1986. The HDV transmission was 12.5% in cases of acute Hepatitis B, 6.7% in homosexual individuals, and 17.8% in drug abusers who were positive for HBsAg. In 1989, the HDV prevalence was found to be 4.9%. In 1996, 0.9% of 923 jaundiced subjects were positive for anti-HDV (18). Since the mid-1990s, however, there has been no new Malaysian data on HBV-HDV co-infection.

Literature review

HDV is an enveloped RNA virus that belongs to the delta viridae family. The Hepatitis B virus is required for the propagation and pathogenicity of the HDV virus (HDV) as the HDV virus is a defective pathogen. HDV can cause acute or chronic hepatitis. Acute Delta Hepatitis may present as simultaneous co-infection with HDV and HBV or HDV superinfection where a person with chronic Hepatitis B is infected by HDV (1).

HDV infection is a major global health challenge. From 2 meta-analyses, about 5-10% of HBV carriers were infected with HDV and up to 60-72 million individuals (13.02 to 14.6% of HBV carriers) had been infected with HDV (2,36,37)

Current treatment of HDV includes Interferon (IFN), nucleotide analogue and combination treatment of interferon with nucleotide analogue (3). IFN requires a long duration of treatment, up to 1 year, with a high dose of IFN needing to be administered (3). A controlled clinical trial in the 1990s showed that interferon alpha is effective in treating HDV; however, relapse is common after completing treatment (32,33). Besides expensive treatment, there are side effects with INF treatment, such as severe lethargy, hyperthyroidism, and reduction in leucocytes, granulocytes, and platelet count (32). As for nucleotide analogue, Famciclovir, Lamivudine, Adefovir And Entecavir have also been assessed for the treatment of chronic HDV. Unfortunately, they all appear ineffective in chronic HDV (3,34). HDV infection can be prevented by Hepatitis B immunisation, but treatment success rates are low.

The HDV virus has been separated into at least eight significant clades based on their genome diversity, with specific geographic distribution (6, 14). HDV-1 is the frequently occurring clade distributed among Europe, the Middle East, America and North Africa (15-18). HDV-2 to HDV-8 occur regionally (19). For instance, HDV-2 prevails in countries like Japan (20), Taiwan (21, 22) and Russia (23). HDV-3, a most diverged genotype, is exclusively found in South America (18, 24, 25). HDV-4 is reported across Japan and Taiwan (21), whereas HDV-5 to HDV-8 are described in Africa (26, 27). The distribution of HDV varies with different geographical regions, with a higher incidence in the Middle East, Mediterranean, Amazonas, Africa, and Asian countries (6, 14, 28-30).

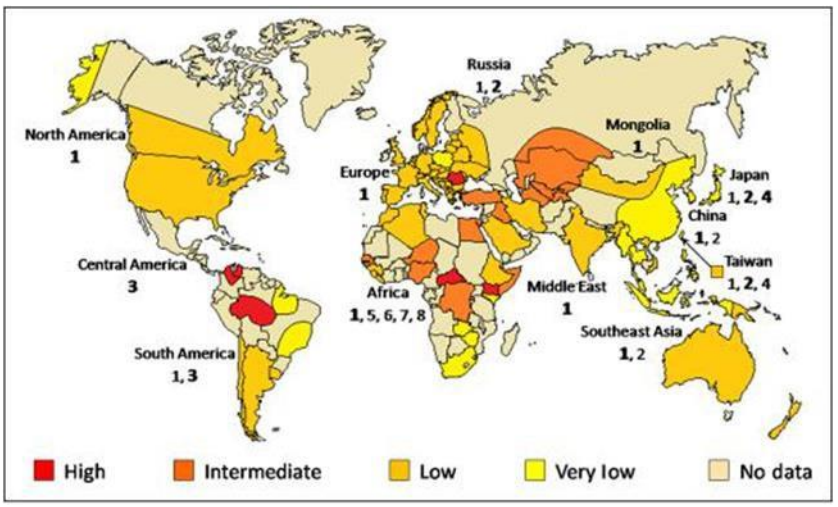

Figure A: Schematic representation of the world's main areas of HDV distribution. Bold numbers represent the predominant HDV genotype for the mentioned area (13)

The study for the distribution of HDV virus in Malaysia was last conducted in 1996, and it showed that 0.9% of 923 jaundiced subjects were positive for anti-HDV. Since then, this has been the only similar study done in Malaysia. In addition, the impact of different genotypes on the clinical manifestation has yet to be established.

Most individuals diagnosed with HDV may progress to liver fibrosis and cirrhosis, developing liver failure or hepatocellular carcinoma (1,2). In addition, subjects with HDV infection also have a high risk of developing hepatocellular carcinoma about sixfold (37). However, the association of HDV virus with liver cirrhosis and liver fibrosis has not been established in Malaysia yet.

According to 2 studies in Taiwan, HDV 1 showed a high association with the development of hepatocellular carcinoma compared to HDV 2 (32.6% vs 11.1%; *P* 0.008). HDV 1 and 2 are the most typical genotypes found in Malaysia (43,44). This information will not only help to provide a further understanding of the molecular epidemiology of HDV in Kelantan and Malaysia, but it may also be helpful to know the association of HDV with clinical parameters, which will help especially physicians in handling and treating subjects.

CHAPTER 2: OBJECTIVES OF STUDY

Objectives of Study

Research Questions

1. What is the prevalence of coinfected HBV-HDV subjects amongst those with chronic HBV at Hospital Universiti Sains Malaysia?
2. What are the HDV genotypes found amongst these coinfected subjects?
3. What are the HBV genotypes found amongst these coinfected subjects?
4. What clinical findings correlate with the presence of HBV-HDV co-infection?
5. What are the clinical findings that correlate with HDV genotypes?
6. What are the clinical findings that correlate with HBV genotypes?

2.1 GENERAL OBJECTIVE

To investigate the epidemiology and clinical characteristics of subjects with chronic Hepatitis B who have concomitant HDV virus (HDV) infection in Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian, Kelantan, Malaysia.

2.2 SPECIFIC OBJECTIVES

Primary

1. To determine HDV prevalence among subjects with Hepatitis B.

Secondary

1. To determine the genetic diversity of HDV virus in these subjects
2. To determine the genetic diversity of the Hepatitis B virus in these subjects

3. To correlate the genotype of HDV virus with the clinical severity of liver disease
4. To correlate the genotype of the Hepatitis B virus with the clinical severity of liver disease

CHAPTER 3: STUDY PROTOCOL

3.1 Study Protocol

Research Title:

Prevalence and Genotype Distribution of HDV Virus Among Chronic Hepatitis B Carriers in Hospital Universiti Sains Malaysia

Conceptual Framework

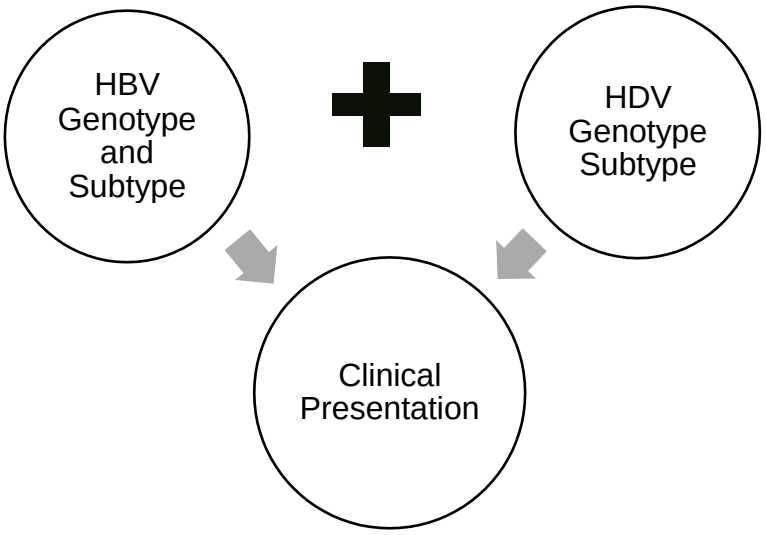

Figure B: Research Conceptual Framework

3.2 Research Design

This was a prospective design, cross-sectional cohort study

Time frame: 1st February 2021 till 27th January 2023

Phase 1

a) Samples and data collection from identified subjects

Phase 2

a) Method optimization/validation
b) Serological analysis of HDV antibody
c) Molecular genotyping of Hepatitis B virus
d) Molecular genotyping of HDV virus

Phase 3

a) Data analysis on prevalence of HDV genotypes and clinical significance

3.3 Study Area

The study was conducted at the Hospital Universiti Sains Malaysia inpatient wards, Gastroenterology Clinic, and the Department of Medical Microbiology & Parasitology, School of Medical Sciences, Universiti Sains Malaysia.

3.4 Study Population

Reference population

Population of Kota Bharu, Kelantan

Target population

The population of Kota Bharu, Kelantan, with a diagnosis of chronic Hepatitis B infection

Source population/sampling pool

The population of Kota Bharu, Kelantan, with a diagnosis of chronic Hepatitis B infection attending Hospital Universiti Sains Malaysia in Kubang Kerian

Sampling frame

Potential subjects will be recruited from the Gastroenterology Clinic appointment list and those admitted to wards that fulfil the inclusion criteria and without exclusion criteria. This study will involve non-probability sampling, i.e, purposive sampling. Subjects who meet the inclusion criteria will be identified and invited to join the study by the researcher.

The purpose of the study will be explained to the subjects, and informed consent will be obtained should they agree to participate. In order to protect patient's private information, each patient will be de-identified and assigned a unique identification

(I.D.) code (for example, HBV001, HBV002). This ID code will be used to label their samples and subsequent work.

3.5 Subject criteria

Inclusion criteria

Age 18 years old or above at the time of recruitment. Having a previous diagnosis of chronic Hepatitis B as evidenced by a positive HBsAg result at or before the recruitment date.

Exclusion criteria

No specific exclusion criteria of note.

3.6 Sample size estimation

According to the World Health Organization (WHO) fact sheet dated Jul 23, 2018, the estimated global prevalence of persons with Hepatitis B who are co-infected with HDV is 5%.

Sample Size Calculation:

$n = (z / \delta)^2 p (1- p)$

p = anticipated population proportion (52.4%)

δ = significance level (0.07)

z = 1.96

Therefore,

$n = (1.96/0.07)^2 0.524(1-0.524)$

$n = (28)^2 \times 0.524 \times 0.476$

$n = 784 \times 0.524 \times 0.476$

n = 196 subjects

Considering 20% additional samples due to possibility of technical errors

$n = 196 + (20 \times 196/100)$

n = 196 + 39

n = 235 subjects

Thus, the total sample size calculated for this study is 235 subjects

3.7 Sampling Method and Subject Recruitment

This study will involve non-probability sampling, i.e, purposive sampling. Subjects who meet the inclusion criteria will be identified and invited to join the study by the researcher. The purpose of the study will be explained to the subjects, and informed consent will be obtained should they agree to participate. In order to protect patient's private information, each patient will be de-identified and assigned a unique identification (I.D.) code (for example, HBV001, HBV002). This ID code will be used to label their samples and subsequent work.

3.8 Research Tool

Table 3.1: Proforma checklist for HDV research

<table>
<tr><td>I.D. code:
(laboratory reference)</td><td></td></tr>
<tr><td>Date of specimen collected:</td><td></td></tr>
<tr><td>Age:</td><td></td></tr>
<tr><td>Ethnicity:</td><td></td></tr>
<tr><td>Employment status:</td><td></td></tr>
<tr><td>Marital status:</td><td></td></tr>
<tr><td>Other infections/diseases:</td><td></td></tr>
<tr><td>Current treatment:</td><td></td></tr>
<tr><td colspan="2">Laboratory investigation</td></tr>
<tr><td>Latest viral load, if available (I.U./uL):</td><td></td></tr>
<tr><td>Latest AST level (Iu/ml):</td><td></td></tr>
<tr><td>Latest ALT level (Iu/ml):</td><td></td></tr>
<tr><td>Presence of liver cirrhosis (Yes/No):</td><td></td></tr>
<tr><td>If liver cirrhosis is present, Child-Pugh score:</td><td>Score:
References:
<table>
<tr><td>2 Minute Medicine®</td><td colspan="2">Child-Pugh Score</td><td>2minutemedicine.com</td></tr>
<tr><td>Factor</td><td>1 point</td><td>2 points</td><td>3 points</td></tr>
<tr><td>Total bilirubin (µmol/L)</td><td><34</td><td>34-50</td><td>>50</td></tr>
<tr><td>Serum albumin (g/L)</td><td>>35</td><td>28-35</td><td><28</td></tr>
<tr><td>PT INR</td><td><1.7</td><td>1.71-2.30</td><td>>2.30</td></tr>
<tr><td>Ascites</td><td>None</td><td>Mild</td><td>Moderate to Severe</td></tr>
<tr><td>Hepatic encephalopathy</td><td>None</td><td>Grade I-II (or suppressed with medication)</td><td>Grade III-IV (or refractory)</td></tr>
</table></td></tr>
</table>

3.9 Data Collection Method

Pre-study preparation

The following items will be prepared prior to patient recruitment:

- Consent forms
- Proforma checklist
- Plain tubes
- Sample label (eg HBV001, HBV002)

Blood and data collection

In this study, a one-off sample collection will be taken from subjects who consented to this study. Blood samples will be taken concurrently with a routine blood test to reduce unnecessary bleeding and its associated risks. Five ml of blood will be collected and kept in plain tubes. These tubes will be labelled with the unique I.D. code assigned to each patient. The blood samples will be promptly sent on ice to the Department of Medical Microbiology and Parasitology and used only for molecular analysis to determine HDV ELISA, HDV PCR and HBV/HDV genotypes.

Demographic and clinical data will be collected directly from the patient or their folder. They may include (but not be limited to) age, sex, race, clinical parameters and current treatment. The informed consent forms and other associated forms or data will be kept in a locked cabinet at the Department of Medical Microbiology and Parasitology. These forms will be destroyed after the study's completion following rules and regulations set by the Jawatankuasa Etika Penyelidikan (Manusia) USM (JEPEM).

Specimens processing

Refer Appendix B

3.10 Study Flowchart

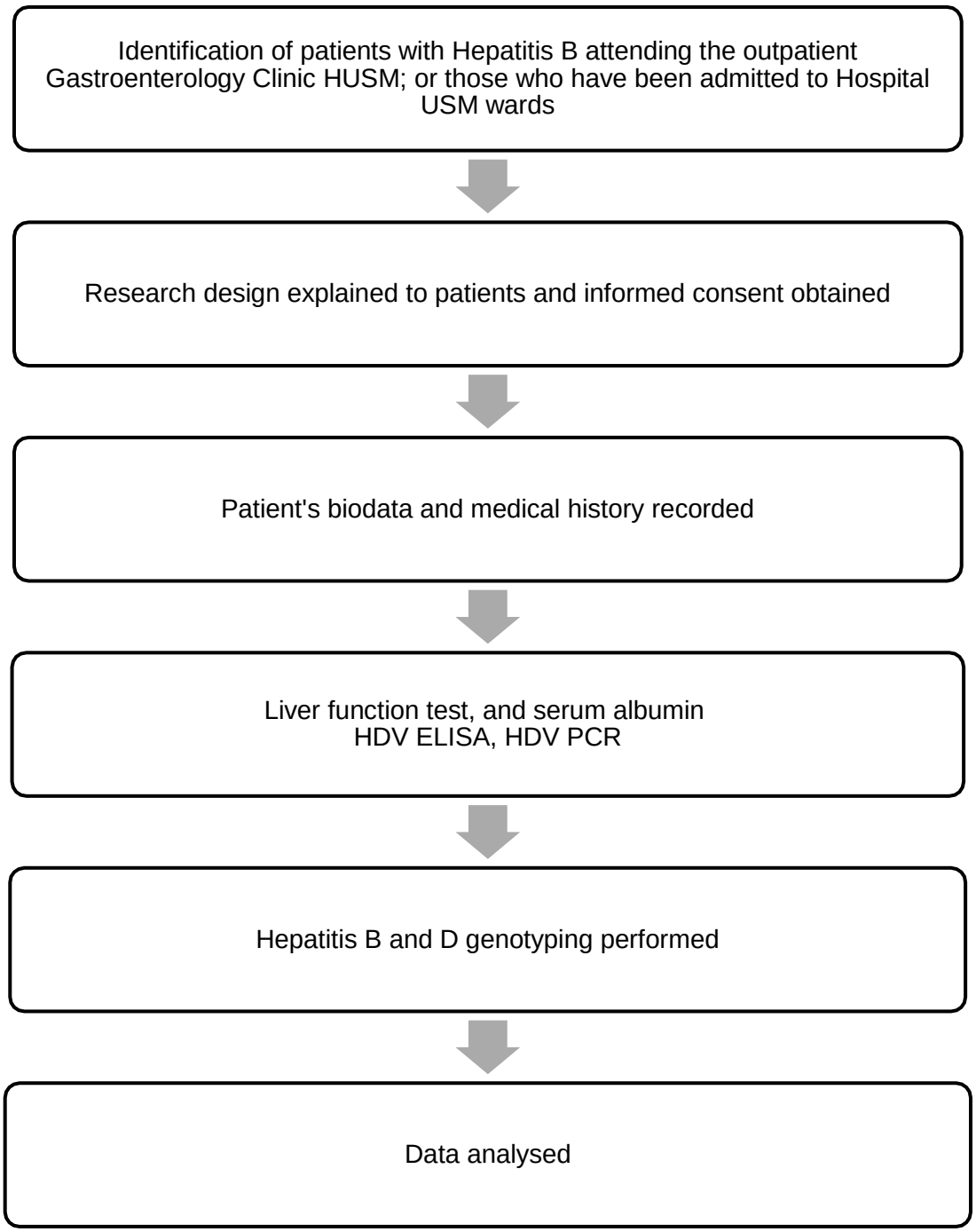

Figure C: Study Flow Chart

3.11 Data Analysis

Statistical analysis plan

Data will be entered and analysed using SPSS version 27. Fisher's exact test will compare categorical data. Non-parametric data will be compared using the Mann-Whitney U test, with a 2-tailed p-value <0.05 considered statistically significant.

Expected result(s)

Table 3.2: Demographic data of Hepatitis B subjects in Hospital USM

Risk factors (n=)	**Prevalence (%)**
Age: <40 40-60 >60	
Ethnicity: Malay Chinese India Others	
Sex: Male Female	

Table 3.3: Laboratory characteristic of Hepatitis B

Clinical outcome (n=)	Prevalence (%)
AST	
ALT	
Liver cirrhosis Yes No	
Child-Pugh score A B C	

Table 3.4: Serological analysis of HDV

Test Outcome (n=)	Prevalence (%)
HDV ELISA HDV PCR	

Table 3.5: Prevalence of Hepatitis B genotype

Hepatits B genotype (n=)	Prevalence (%)
Genotype B Genotype C Genotype D Others	

Table 3.6: Prevalence of HDV genotype

HDV genotype (n=)	Prevalence (%)
Genotype 1 Genotype 2 Genotype 4 Others	

3.12 Gantt Chart & Milestone

Milestone	Duration										
	Feb-21	Mar - 21	Apr - 21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21
Sample and data collection											
Serological analysis of HDV ELISA											
Optimisation of primers and PCR											
Viral DNA/RNA extraction and cDNA synthesis											

Milestone	Duration												
	Jan – 22	Feb - 22	Mar - 22	Apr - 22	May - 22	Jun - 22	Jul - 22	Aug – 22	Sep - 22	Oct - 22	Nov - 22	Dec - 22	Jan - 23
Sample and data collection													
Molecular genotyping of the Hepatitis B virus													
Molecular genotyping of the HDV virus													
Phylogenetic analysis													
Data analysis and report submission													

Table 3.7: Milestone Descriptions

	Milestone Description *Perincian Perbatuan*	**Schedule** *Perancangan*	
		Month *Bulan*	**Year** *Tahun*
M1	Sample and data collection	Feb	2021
M2	Serological analysis of HDV ELISA	March	2021
M3	Optimisation of primers and PCR	May	2021
M4	Viral DNA/RNA extraction and cDNA synthesis	Sept	2021
M5	Molecular genotyping of the Hepatitis B virus	Jan	2022
M6	Molecular genotyping of the HDV virus	May	2022
M7	Phylogenetic analysis	Sept	2022
M8	Data analysis and report submission	Dec	2022

Budget proposal

Grant type application: Research University - Individual

Approved: 1st May 2021 – 30th April 2024

Budget allocation: RM54 000

List of investigators and role

Table 3.8: List of Investigator and Role

Investigators	**Role**	**Location of study**
Dr Fathin Hadi	Principal Investigator	Hospital Universiti Sains Malaysia
Assoc. Prof Dr Nazri Mustaffa (MMC:	Supervisor	Hospital Universiti Sains Malaysia
Assoc. Prof Dr Rafidah Hanim Shueb	Supervisor	Hospital Universiti Sains Malaysia
Dr Wong Mung Seong	Sub Investigator at the site	Hospital Universiti Sains Malaysia
Mr Bello Kizito E	Sub Investigator at the site	Hospital Universiti Sains Malaysia
Dr Vincent Tee Wei Shen	Sub Investigator at the site	Hospital Universiti Sains Malaysia
Dr Ummu Atikah Zulkifli	Sub Investigator at the site	Hospital Universiti Sains Malaysia