SEROPREVALENCE OF HEPATITIS B IN FIRST- TIME BLOOD DONORS OF NATIONAL BLOOD CENTRE AFTER IMPLEMENTATION OF NATIONAL VACCINATION PROGRAMME

BY

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DECLARATION

I hereby declare that this research has been sent to Universiti Sains Malaysia for the degree of Masters of Medicine in Transfusion Medicine. It is not to be sent to any other universities. With that, this research might be used for consultation and can be photocopied as reference.

DR. EMMY SOW

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LIST OF ABBREVIATIONS

Anti-HBc	Anti-hepatitis B core antigen
Anti-HBe	Anti-hepatitis B e antigen
Anti-HBs	Anti-hepatitis B surface antigen
BBIS	Blood Bank Information System
CLIA	Chemiluminescent immunoassay
DNA	Deoxyribonucleic acid
DTP	Diphtheria–Tetanus–Pertussis
ECL	Electrochemiluminescent immunoassay
EIA	Enzyme immunoassay
HA	Haemagglutination
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma

HCV	Hepatitis C virus
Hib	*Haemophilus influenzae type b*
HIV	Human Immunodeficiency Virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IPV	Inactivated polio vaccine
LFT	Liver function test
MOH	Ministry of Health Malaysia
MSM	Men who have sex with men
NAT	Nucleic acid testing
NBC	National Blood Centre of Malaysia
OBI	Occult blood infection
PA	Particle agglutination
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
SOP	Standard Operating Procedure
TMA	Transcription-mediated amplification

TML	Transfusion microbiology
TTI	Transfusion transmitted infection
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

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ABSTRAK

Seroprevalens Jangkitan Hepatitis B di Kalangan Penderma Darah Kali Pertama Pusat Darah Negara Selepas Pelaksanaan Program Vaksinasi Nasional

Latar belakang: Prevalen hepatitis B di Malaysia adalah kira-kira 5% dengan kadar mortaliti yang berkaitan 0.04 pada tahun 2013. Langkah-langkah pencegahan penyebaran penyakit dilaksanakan menerusi program vaksinasi kebangsaan sejak tahun 1989 dengan liputan vaksin sebanyak 99.3%. Di Malaysia, ujian saringan penderma darah untuk virus Hepatitis B (HBV) dengan kaedah serologi telah dilaksanakan sejak tahun 1974 manakala ujian asid nukleik (NAT) bermula sejak tahun 2007 di Pusat Darah Negara.

Objektif: Untuk mengenalpati seroprevalen jangkitan hepatitis B di kalangan penderma darah kali pertama selepas pelaksanaan program vaksinasi nsional untuk hepatitis B.

Kaedah: Kajian rentas retrospektif yang meneliti rekod 215 orang penderma darah kali pertama yang merupakan warganegara Malaysia yang disaring positif untuk HBV dan menderma antara 1 Januari 2010 hingga 31 Disember 2015 di Pusat Darah Negara.

Keputusan: Prevalen HBV di kalangan penderma kali pertama NBC dari 1 Januari 2010 ke 31 Disember 2015 adalah 0.15%. Prevalen HBV di kalangan penderma dilahirkan

sebelum tahun 1989 (era pra-vaksinasi) adalah lebih tinggi pada 0.23% berbanding 0.05% di kalangan penderma yang lahir pada dan selepas tahun 1989 (era pos-vaksinasi). Lebih ramai penderma yang dilahirkan pada era pra-vaksinasi mempunyai faktor risiko berbanding penderma kumpulan kedua. Bagi peringkat jangkitan HBV, paling ramai penderma adalah pada peringkat tidak mereplikasi diikuti peringkat mereplikasi dan peringkat penyembuhan. Bagi penderma dari era pra-vaksinasi, majoriti penderma (97.2%) adalah HbsAg+ dan semua adalah anti-HBc+, 30.3% adalah HbeAg+ dan 71% adalah anti-Hbe+. Bagi penderma dari era pos-vaksinasi, 98.6% penderma adalah HbsAg+ dan anti-HBc+, 50% adalah HbeAg+ dan 48.6% adalah anti-Hbe+. Semua penderma adalah reaktif untuk ujian NAT tanpa mengira tahun kelahiran. Terdapat perkaitan antara seroprevalen HbeAg dan anti-Hbe dengan tahun kelahiran ($p<0.05$). Keputusan menunjukkan bahawa tiada perkaitan antara peringkat jangkitan dengan faktor risiko penderma dan ciri-ciri demografi mereka ($p>0.05$).

Kesimpulan: Prevalen HBV di kalangan penderma yang menerima vaksinasi semasa lahir adalah rendah daripada mereka yang tidak menerima vaksinasi. Jangkitan *occult* (anti-HBc+, NAT+ dengan HBsAg-) juga lebih rendah di kalangan penderma yang menerima vaksinasi sewaktu lahir. Pelaksanaan program vaksinasi nasional HBV telah menyumbang kepada pengurangan jangkitan HBV dan mengurangkan risiko transmisi yang berkaitan dengan transfusi darah.

Kata kunci: hepatitis B, penderma darah, vaksinasi, seroprevalen

ABSTRACT

Seroprevalence of Hepatitis B in First Time Blood Donors of National Blood Centre after Implementation of National Vaccination Programme

Background: Malaysia's prevalence of hepatitis B is about 5% with the related mortality rate of 0.04 in 2013. Transmission preventive measures were implemented via national vaccination programme since 1989 with vaccine coverage of 99.3%. In Malaysia, universal blood donor screening for hepatitis B virus (HBV) using serology method was implemented since 1974 while nucleic acid testing (NAT) began since 2007 in National Blood Centre.

Objective: To determine the seroprevalence of hepatitis B among first-time blood donors after implementation of national hepatitis B vaccination programme.

Methods: A cross sectional study involving review of 215 Malaysian first time donors' records that was screened positive for HBV and donated between 1st January 2010 to 31st December 2015 at National Blood Centre.

Results: The overall prevalence of hepatitis B among the first-time blood donors of NBC from 1st January 2010 to 31st December 2015 was 0.15%. The prevalence of hepatitis B

was higher at 0.23% among donors born before year 1989 (pre-vaccination era) compared to 0.05% among donors born in and after year 1989 (post-vaccination era). More donors born pre-vaccination era have known risk factors compared to the other group of donors. The stages of infection were highest for non-replicating followed by replicating and convalescence regardless year of birth. Among donors born pre-vaccination era, majority (97.2%) were HBsAg+, all were anti-HBc+, 30.3% were HBeAg+ and 71% were anti-HBe+. For donors born post-vaccination era, 98.6% were HBsAg+ and anti-HBc+, 50% were HBeAg+ and 48.6% were antiHBe+. All donors were NAT+ irrespective of year of birth. There was significant association of the seroprevalence for HBeAg and anti-HBe with year of birth ($p < 0.05$). There was no association between stages of infection with risk factors and demographic characteristics of donors ($p > 0.05$).

Conclusions: The prevalence of HBV among donors who received birth vaccination was lower than those who did not. Occult infection (anti-HBc+, NAT+ with HBsAg-) was also reduced in donors who received birth vaccination. Implementation of national vaccination programme for HBV infection has contributed to the reduction of HBV infection and in minimizing the risk of transfusion-related transmission.

Keywords: hepatitis B, blood donors, vaccination, seroprevalence

CHAPTER 1

INTRODUCTION

1.1 Overview of Hepatitis B

Hepatitis B is a vaccine-preventable infection caused by hepatitis B virus (HBV) of the hepadnaviridae family which was discovered in 1965 by Dr Baruch Blumberg (CEVHAP, 2012). It is an enveloped deoxyribonucleic acid (DNA) virus and has various antigenic components consisting of hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg), and hepatitis B e antigen (HBeAg) (Murphy T. *et al.*, 2015).

It can be classified into 10 genotypes (A to J) and the prevalence of these genotypes varies geographically as listed below (Norder H. *et al.*, 1993; Lindh M. *et al.*, 1997; Liu C.J. and J.H., 2013).

i. Genotype A is found mainly in Northern Europe, North America, India, and Africa.
ii. Genotype B and C are prevalent in Asia.
iii. Genotype D is more common in Southern Europe, the Middle East, and India.
iv. Genotype E is restricted to West Africa.
v. Genotype F is found in Central and South America.
vi. Genotype G has been reported in France, Germany, and the United States of America (USA).
vii. Genotype H is found in Central America.

viii. Genotype I is found in Vietnam and Laos.

ix. Genotype J is found in the Ryukyu Islands in Japan.

As for Malaysia (being part of Asia), the prevalent HBV genotypes are mainly genotype B and C. The heterogeneity occurs due to lack of proofreading activity during reverse transcription of the pre-genomic ribonucleic acid (RNA) causing mutation during the process of viral replication. The rate of mutation is estimated to be about $2x10^4$ base substitutions/site/year, about 100 times higher than other DNA viruses but about 100 to 1000 times lower than other RNA viruses (Girones R. and R., 1989). Reports suggested that HBV genotypes may influence clinical outcomes, HBeAg seroconversion rates, mutational patterns in the pre-core and core promoter regions, and response to interferon therapy (Lok, 2017).

The virus can be transmitted via parenteral or mucosal exposure to infected body fluids (Murphy T. *et al.*, 2015). Therefore, blood donors who are infected be it acute or chronic, can transmit the infection to patients who received the infected blood products. After the discovery of the virus, Dr Blumberg together with microbiologist Irving Millman managed to develop a blood test for this virus in the year 1969 and blood banks started using the test to screen donated blood for hepatitis B in the year 1971 (CEVHAP, 2012). With the availability of screening test, the incidence of transfusion-transmitted hepatitis B infection showed a marked reduction of 25% (Alter H. J. *et al.*, 1972).

Hepatitis B infection can be acute or chronic and has a wide range of presentation. Infected individuals can be asymptomatic or present as symptomatic progressive disease. This infection is significant because it is strongly associated with hepatocellular carcinoma (HCC) and liver cirrhosis. It has been reported that 50% of HCC is caused by hepatitis B (Perz J. F. *et al.*, 2006). World Health Organization (WHO) also reported that in the year 2015, an estimated of 887,000 mortality worldwide is due to hepatitis B-associated acute and chronic liver disease (WHO, 2017c). Meanwhile, in Malaysia, the prevalence of hepatitis B is about 5% (APAVH, 2012) with hepatitis B-related mortality rate of 0.04 in the year 2013 (MOH, 2014b).

Due to the severity of the complications, some measures have been taken to prevent the transmission of the virus. One of the measures introduced is vaccination. Since the year 1989, the expanded programme on immunisation where all neonates are given hepatitis B vaccination is being implemented in Malaysia. The vaccination is given the first dose at birth, the second dose at 1-month-old and the third dose at 5 months old (Wong S. L. *et al.*, 2002).

1.2 Hepatitis B in blood donors

Most of the blood donors with HBV infection are asymptomatic. A number of them do not reveal their past history of infection or if they were exposed to any risk factors. Among the reported reasons were that they thought the fact was irrelevant, they do not feel comfortable or embarrassed to discuss the risk factors and that they felt healthy (Ferreira and Passos, 2012). These donors were then detected to be hepatitis B positive from the screening tests either serology or NAT test or both.

In Malaysia, the universal screening of all blood donors for hepatitis B has been implemented by the blood transfusion services since the year 1987 (MOH, 2016a). It started with serological screening test. Then in the year 2007, nucleic acid testing (NAT) was introduced in the central region by National Blood Centre (NBC) in Kuala Lumpur (MOH, 2010). The NAT services were then expanded to all the states except Penang, Perak, Sabah and Sarawak by the year 2016 (MOH, 2016a). All the donated blood, plasma or platelets products will be screened and if screening is positive, the donors will be traced and contacted for further investigations. At the same time, they will be deferred from future donation and all the donated blood products will be discarded. A look-back and recall procedure will also be initiated for seroconverted donors (NBC, 2014).

Based on NBC's working instruction, PDN/WI-04 Version No. 05 2nd January 2014, a formal letter will be sent to all blood donors with positive screening results. They will be requested to come to NBBC for further counselling and investigations. When a donor

comes to NBC for counselling, relevant history and risk factors for HBV infection will be elicited. Blood will be taken for repeat testing of HBsAg and additional HBV serology markers such as anti-HBe, HBeAg, anti-HBs (if HBsAg negative with positive NAT) and liver function test (LFT). An appointment date will then be given to the respective donors to review the results. During the second counselling visit, if the results show confirmed positive for HBV, the donors will be counselled and are required to sign a donor declaration form stating that the donor understands their condition and agree not to donate their blood in the future. The donor will then be permanently deferred from blood donation and referred to Gastroenterology (Medical) Clinic in Hospital Kuala Lumpur. Electronic-notification to the local health authorities will also be carried out. All the clinic reviews, investigations and results will be documented and recorded in the donors' cards and in the Blood Bank Information System (BBIS) (NBC, 2014).

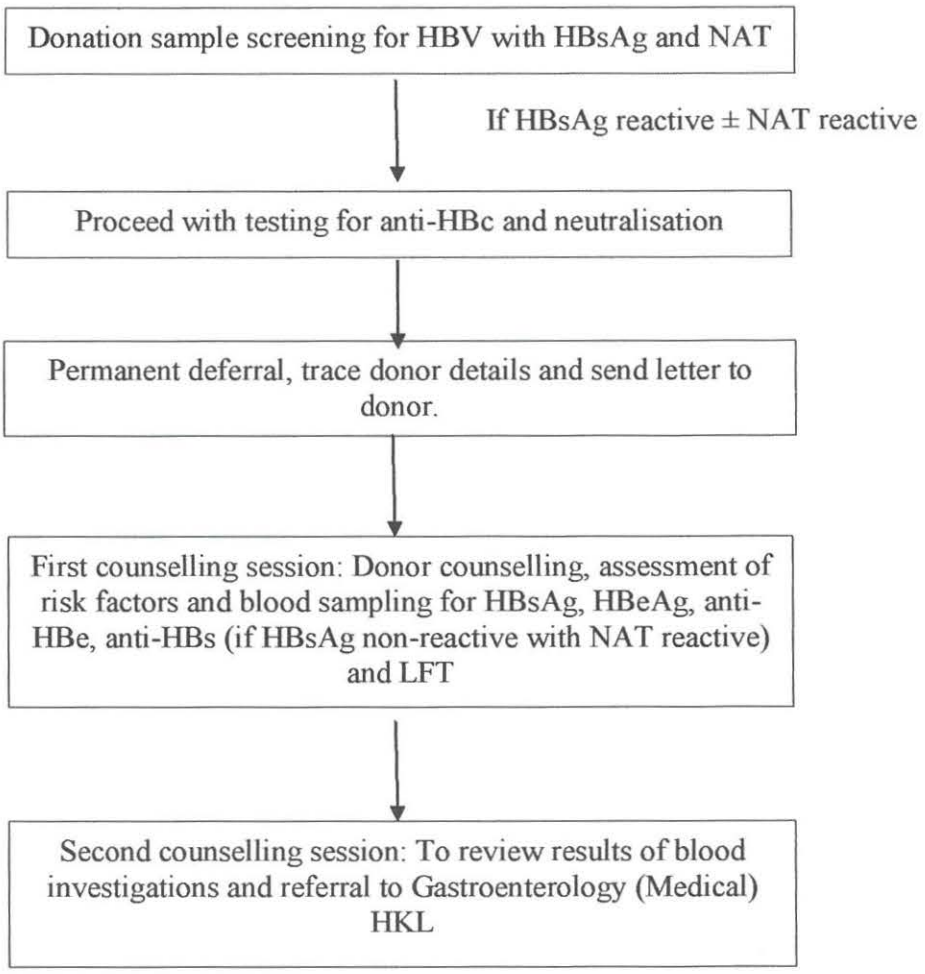


Figure 1: Flow of HBV screening in blood donors of NBC

1.3 Research justification and benefits

In Malaysia, HBV infection is still one of the main reasons for permanent deferral of donors. Permanent deferral due to hepatitis B was reported to be 66.6%, 68.6%, 64.6%, 62.3%, 61.6% and 58.1% for year 2010 to year 2015 respectively out of the total permanent deferrals due to transfusion-transmitted infections (TTI) (MOH, 2011; MOH, 2012; MOH, 2013; MOH, 2014a; MOH, 2015; MOH, 2016a). Therefore, the determination of associated risk factors will aid in the measures to improve the safety of blood and blood products. By identifying the associated risk factors, donor counselling can be improved.

The determination of the efficiency of hepatitis B vaccine for prevention of transmission can aid in future measures to further minimise the incidence of hepatitis B such as booster dose after 5 years. A study reported that most vaccine-induced production of anti-HBs levels are protective for five to ten years after vaccination although definite duration remains undefined (Krajden M. *et al.*, 2005).

Screening tests for HBV started initially with serological method since the year 1971 (CEVHAP, 2012). With the advancement of technology, molecular methods were then developed. A molecular method such as NAT aids in the detection of HBV during window period and Occult Blood Infection (OBI) (Allain J.P. *et al.*, 2013; Hans R. and N., 2014). This group of donors will usually be detected only with NAT and at present, not all blood collection centres are utilising NAT screening. There are some laboratories which are still

using HBsAg as serology marker for screening of HBV, in which the diagnosis of OBI will be missed. This is important because, there is a high risk of hepatitis B transmission by the transfusion of blood products from donors with OBI (Allain J.P. *et al.*, 2013).

1.4 Hypothesis

1.4.1 Research hypothesis

i. The seroprevalence of hepatitis B infection among first-time blood donors born post-national hepatitis B vaccination programme is reduced compared to first-time blood donors born pre-national hepatitis B vaccination programme.

ii. There is an association between the stage of hepatitis B infection in blood donors with risk factors and demography data.

1.4.2 Null hypothesis

i. The seroprevalence of hepatitis B infection among first-time blood donors born post-national hepatitis B vaccination programme is similar compared to first-time blood donors born pre-national hepatitis B vaccination programme.

ii. There is no association between the stage of hepatitis B infection in blood donors with risk factors and demography data.

1.5 Research Objectives

1.5.1 General Objective

To determine the seroprevalence of hepatitis B among first-time blood donors after implementation of national hepatitis B vaccination programme.

1.5.2 Specific Objectives

i. To determine the prevalence of hepatitis B infection among first-time blood donors born pre-and post-national hepatitis B vaccination programme.

ii. To describe the demographic data, risk factors and stages of hepatitis B infection upon detection among the reactive first-time blood donor donors born pre- (before the year 1989) and post- (born in or after the year 1989) national hepatitis B vaccination programme.

iii. To compare the seroprevalence of hepatitis B infection among first-time blood donors born pre-and post-national hepatitis B vaccination programme.

iv. To determine the association between the stages of infection with risk factors and demography data.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

It was reported by WHO that in the year 2015, 1.34 million deaths were due to viral hepatitis. This is comparable to deaths due to tuberculosis and higher than HIV-related mortality. The viral hepatitis-related mortalities were due to chronic liver disease with 720,000 deaths due to cirrhosis and 470,000 deaths due to HCC (WHO, 2017a). Globally, in the same year, it was estimated that there were 257 million people who were living with chronic HBV infection. The affected regions were mostly the African Region and the Western Pacific Region (WHO, 2017c).

Malaysia, categorised as part of the Western Pacific region reported its hepatitis B infection incidence rate of 12.65 per 100,000 population and mortality rate of 0.13 for the year 2015 (MOH, 2016b).

2.2 Risk factors for hepatitis B infection

Even though HBV can be found in all body fluids, secretions and excretions but the risks of transmission are only via percutaneous and mucosal exposure to infected blood, body fluids containing visible blood, semen and vaginal secretions (CDC 2001). The modes of transmission include sexual or close household contact with an infected person, vertical transmission (mother to infant), injecting drug use and nosocomial exposure (Wormser G.P. and R.L., 2008). Percutaneous exposures that have resulted in HBV transmission include transfusion of unscreened blood or blood products, sharing unsterilised injection needles for intravenous drug use, haemodialysis, acupuncture, tattooing and needle-stick injuries sustained by hospital personnel (WHO, 2002).

Therefore, the possibilities of donors being exposed to these risks are included in the pre-donation questionnaire and are explored in depth during pre-donation counselling sessions. Donors who have any of these risks are deferred from donation to ensure blood safety (NBC, 2016).

2.3 Transfusion-transmitted hepatitis B

Transfusion of contaminated blood products had been reported to transmit HBV. Comparatively, the risk of HBV transmission is 100 times higher than HIV. The risk of transmission of HBV per exposure is 52 % - 69% if a person is transfused with HBsAg reactive blood (CDC, 1991). As for NBC, the residual risk for hepatitis B for the year 2016 is 5.26 per million donations or 1 in 190,207 donations (NBC, 2017b).

Meanwhile in the United Kingdom (UK), reported since the year 1996, there were 30 cases of confirmed transfusion-transmitted viral infections involving 37 recipients. Hepatitis B is the most common proven cause of viral TTI. This occurrence is possible where a recently infected donor donates during window period and the infection is not detected by the screening tests despite using NAT for testing (Bolton-Maggs P.H.B. and D., 2017).

2.4 Significance of the hepatitis B serologic markers

HBV is a small double-shelled virus with various antigenic components. These components contribute to the confusion in interpretation of hepatitis B serology because of a large number of different potential serological profiles. The presence of different combinations of serologic markers serves as a guide for interpretation of the possible stage of infection. The serologic markers consist of HBsAg, anti-HBs, anti-HBc (IgG, IgM and total), HBeAg and anti-HBe (Watson, 2008).

HBsAg is a protein on the surface of the HBV. It can be detected in serum during acute or chronic HBV infection (if it persists more than 6 months in duration). The presence of the antigen is indicative that the individual is infectious. After being infected, the body will produce antibody as part of the immune response against this antigen which is named anti-HBs. The presence of this antibody usually indicates recovery and immunity from HBV. Besides infection, anti-HBs is also produced in vaccinated individuals. However, the antibody level reduces over time and can become undetectable (Watson, 2008; CDC, 2012a).

Meanwhile, HBeAg is a secreted product of the nucleocapsid gene of the virus that is found in serum during both the acute and chronic phase of infection. Its presence indicates that the virus is replicating, and the infected individual has high levels of HBV. During acute or after a burst of viral replication, the body's immune system will produce antibody to the HBeAg. The spontaneous conversion from e antigen to e antibody (also known as seroconversion) is a predictor of long-term clearance of HBV in patients undergoing antiviral therapy and indicates lower levels of HBV (Watson, 2008; CDC, 2012a).

For total anti-HBc, it appears at the onset of symptoms of acute hepatitis B and persists for life. Therefore, its presence indicates previous or ongoing infection with HBV in an undefined time frame. There is a component of IgM anti-HBc and positivity of this marker indicates recent infection with HBV (less than 6 months) and is usually indicative of acute infection. On the other hand, IgG anti-HBc positivity indicates chronic hepatitis B (6

months or more) (Watson, 2008; CDC, 2012a). In NBC, the anti-HBc measured is the total anti-HBc. Therefore, it is difficult to determine whether a donor is acutely or chronically infected based only on total anti-HBc. Table 2.1 and Table 2.2 show the interpretation of the serology markers and the possible stage of infection (Robinson W. S., 1995; Hollinger F. B. and J., 2001).

Table 2.1: From the reaction of serology markers, the possible stage of infection that the donor was in during testing can be determined (Hollinger F. B. and J., 2001)

Assay results			**Interpretation**
HBsAg	**anti-HBs**	**anti-HBc**	
+	-	-	Early acute HBV infection
+	+/-	+	Acute or chronic HBV infection that can be differentiated with IgM anti-HBc. Level of infectivity can be determined with HBeAg or HBV DNA.
-	+	+	Indicates previous HBV infection and immunity to HBV.
-	-	+	Possibilities include: a) Resolved infection (most common) b) False-positive anti-HBc c) "Low level" chronic infection d) Resolving acute infection
-	+	-	Vaccine-type response.

Table 2.2: Serological test findings at different stages of HBV infection and in convalescence (Robinson W. S., 1995)

Stage of infection	HBsAg	anti-HBs	anti-HBc		HBeAg	anti-HBe
			IgG	IgM		
Late incubation period	+	-	-	-	+/-	-
Acute HBV infection or persistent carrier state	+	-	+	+	+	-
HBsAg-negative acute hepatitis B	-	-	-	+	-	-
Recovery with loss of detectable anti-HBs	-	-	+	-	-	-
Healthy HBsAg carrier	+	-	+++	+/-	-	+
Chronic hepatitis B, persistent carrier state	+	-	+++	+/-	+	-
Hepatitis B infection in recent past, convalescence	-	++	++	+/-	-	+
Hepatitis B infection in distant past, recovery	-	+/-	+/-	-	-	-
Recent hepatitis B vaccination, repeated exposure to antigen without infection, or recovery from infection with loss of detectable anti-HBc	-	++	-	-	-	-

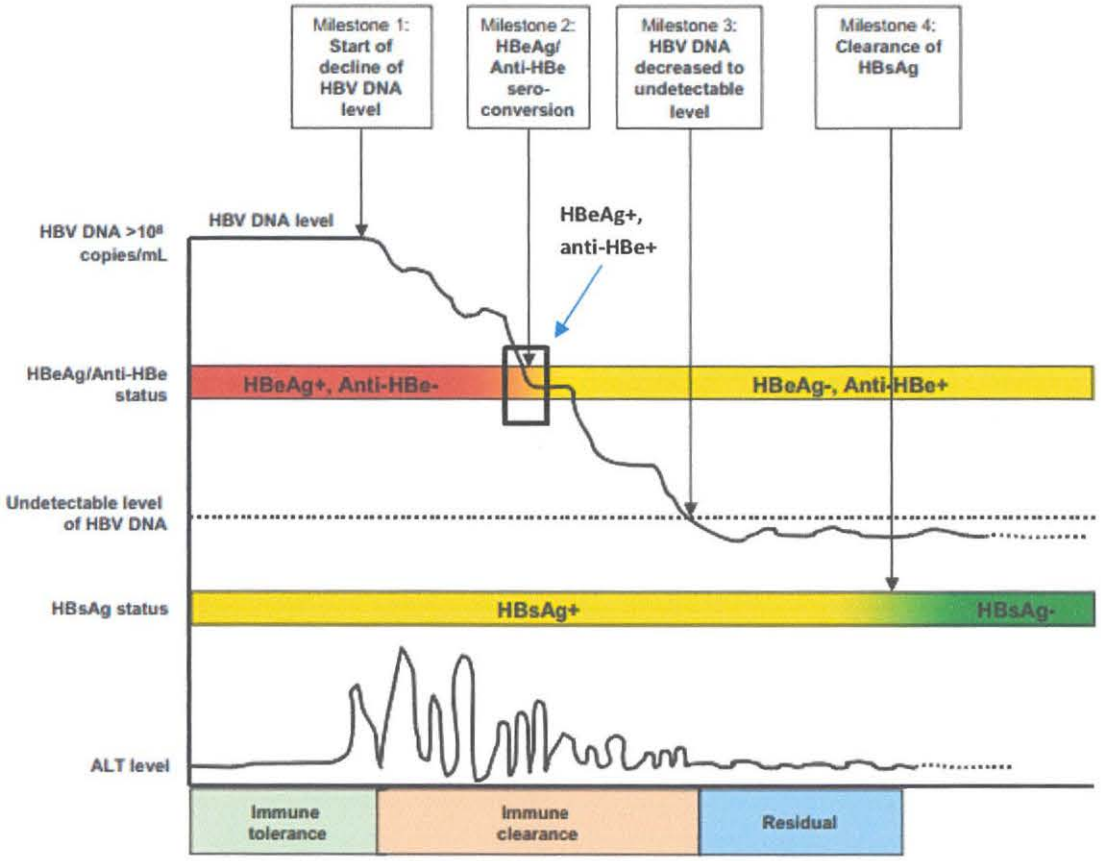


Figure 2.1: The milestones of HBV markers based on the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus (REVEAL-HBV) Study findings (Chen C. J. and I., 2011).

2.5 Serologic testing methods for detection of HBV infection

There are various assays that have been developed for blood screening. These assays detect the host immune response (antibodies), the viral antigens or the nucleic acid of the infectious agent. The main assays used are immunoassays and NAT (WHO, 2010).

Immunoassays are based on the ability to detect antibody, antigen or a combination of the two which are inclusive of enzyme immunoassays (EIAs), chemiluminescent immunoassays (CLIAs), electrochemiluminescent immunoassays (ECLs) hemagglutination (HA) or particle agglutination (PA) assays and rapid tests. ECL and CLIA are variations of EIA. For HBV testing, the assays usually being used are NAT, EIA, ECL, CLIA and neutralisation tests (WHO, 2010; WHO, 2017b).

The process of EIA utilises the basic immunology concept of antigen-antibody binding. Therefore in CLIA, enzymes used will convert a substrate to a reaction product with light emission via the chemical reaction while in ECL, the chemiluminescent reactions that lead to the emission of light are initiated electrically (Mathew B. C. *et al.*, 2005; Wang C. *et al.*, 2012).

In NBC, the assays performed for HBV testing are EIA, ECL and CLIA (serology), NAT and neutralisation test as a confirmatory test. Hepatitis B infection is confirmed if observed antigen reactivity is neutralised upon repeat testing where the neutralisation reagent can abolish reactivity in the assay in comparison with a control reaction (WHO, 2017b).

2.6 Role of NAT test

Despite the availability of screening test for HBV, there were still a substantial number of cases of transfusion-transmitted hepatitis B. This is attributed to the serologic window period that is defined as the time between potential exposure to HBV and the point when the test can detect the infection (Bolton-Maggs P.H.B. and D., 2017). However, with the development and use of NAT, this risk has been reduced (Busch, 2004; Dettori S. *et al.*, 2009; Stramer S. L. *et al.*, 2011; Hans R. and N., 2014)

NAT is a molecular testing technique that is highly sensitive and specific for viral nucleic acids. Thus, the window period for hepatitis B infection had been reduced from 59 days to about 10 days (Weusten J. *et al.*, 2011). Besides the benefit of narrowing the window period, it has the additional benefits of resolving biological false reactive results and in detecting OBI in hepatitis B. OBI is defined as the presence of HBV DNA in the blood or liver tissues in HBsAg negative with or without any HBV antibodies (Allain, 2004; Mitri M.S.D. *et al.*, 2010).

Romano and colleagues reported that among the first-time blood donors in Italy, 8.3% was reactive for anti-HBc and 0.32% were reactive concurrently with HBsAg. Among this group of donors, 86.7% of them had immunity or anti-HBs reactive. Meanwhile, 10.4% of the donors were reactive for anti-HBc only. The presence of isolated anti-HBc was suggestive of occult infection and HBV DNA was detected (Romano L. *et al.*, 2013). This

shows that screening test using HBsAg still has risks of missed detection as shown in the data that there is circulating HBV DNA even if HBsAg is not detected.

The technologies commonly used for NAT testing are polymerase chain reaction (PCR), and transcription-mediated amplification (TMA) (Strobi, 2011). PCR is a technique that amplifies a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. Meanwhile, TMA is a technique that utilises two enzymes, ribonucleic acid (RNA) polymerase and reverse transcriptase, to rapidly amplify the target RNA or DNA, producing hundreds of millions of copies of the targeted sequences (Strobi, 2011). TMA has the benefits of not requiring washing steps and no transfer of amplicon out of the tube. Hence, it simplifies the procedure and reduces risks of contamination (Bioscience, 2016). In NBC, the NAT testing is performed via the TMA technique.

2.7 Role of universal hepatitis B vaccination

There are two types of hepatitis B vaccines which consist of plasma-derived and recombinant. The plasma-derived vaccines which were first commercially available in the year 1982, were developed using purified HBsAg from the plasma of chronically-infected HBV patients. By the year 1986, the recombinant hepatitis B vaccine became available and gradually replaced the plasma-derived hepatitis B vaccines (Mast E. and J., 2008). Both the types of vaccines show no difference in terms of efficacy, reactogenicity and duration of protection (WHO, 2009b). These vaccines confer active immunity via formation of anti-HBs against the HBsAg present in the vaccines either produced through recombinant DNA techniques from yeast cells or purified from plasma of chronically-infected hepatitis B patients (WHO, 2012). Recombinant hepatitis B vaccines are available in both monovalent formulations for birth doses or for adult vaccination and in combination with other vaccines for paediatric vaccination such as diphtheria–tetanus–pertussis (DTP), *Haemophilus influenzae type b* (Hib), and inactivated polio vaccine (IPV) (Mast E. and J., 2008). Vaccine-induced antibody had been shown to persist for at least 10 to 15 years and the duration of anti-HBs is related to the antibody peak level achieved after the primary vaccination (Zanetti A. R. *et al.*, 2008).

In Malaysia, Ng and colleagues reported that the overall HBsAg prevalence among University of Malaya students is 0.62% with 1.08% in students born before 1989 and 0.2% in students born in or after 1989. They also reported that by year of testing, HBsAg prevalence declined steadily from 1.27% (2005) to 0.49% (2009) and 0% (2010 and 2011). In addition to that, he also reported that 66.14% of those vaccinated during infancy had no

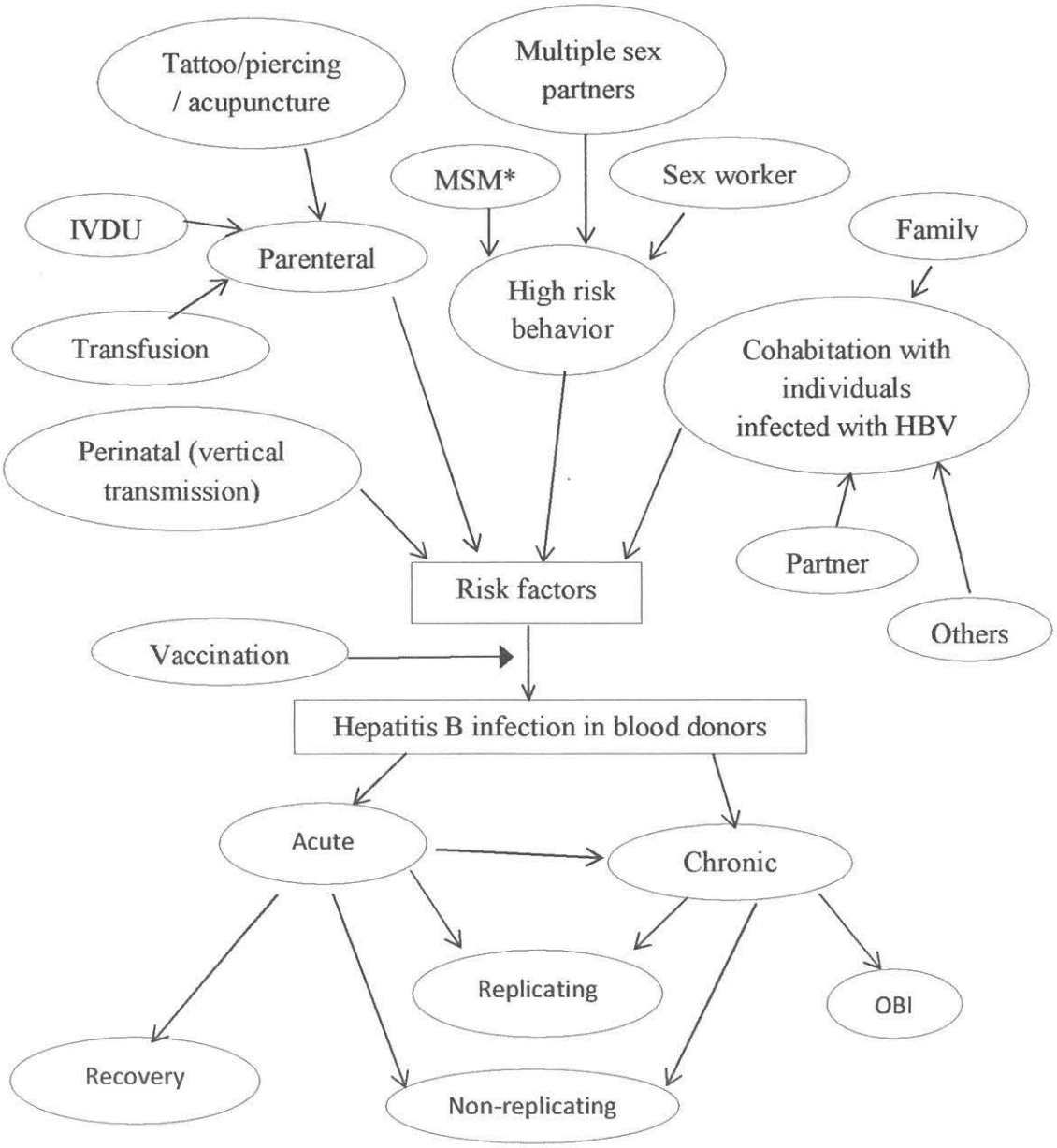
demonstrable immunity at the time of screening with 0.39% have anti-HBc, including the three students who were HBsAg positive (Ng K.P. *et al.*, 2013). This indicates that the antibody level in the body reduces with time and despite the vaccination at birth, there are still risks of HBV infection.

According to Mele and colleagues, the incidence of acute hepatitis B from the year 1991 to 2005 showed reduction of 24-fold (15-year to 24-year age group), 50-fold (0-year to 14-year age group) and the incidence decreased by half for the 25-year age group after the implementation of universal vaccination in Italy. Among the patients with acute hepatitis B, it was documented that they were high-risk individuals who had missed immunisation with 50% of them had history of cohabitation with hepatitis B carriers and 70% of them were injection drug users. This study also reported that the strongest associations with acute hepatitis B were due to blood transfusion followed by cohabitation with hepatitis B carriers, injection drug use and unsafe sexual practices (Mele A. *et al.*, 2008).

Taiwan, similar to Malaysia, also has a universal newborn hepatitis B immunisation programme. Su *et al.* (2012) had reported that the highest incidence rates of acute hepatitis B occurred among unvaccinated individuals aged between 25 to 39 years (2.33/100000). The incidence in vaccinated birth cohorts was significantly lower than in unvaccinated birth cohorts among patients between 15 years to 24 years old, with an adjusted-relative risk of 0.42 (Su W. J. *et al.*, 2012). This study showed that hepatitis B vaccination does offer protection against acute hepatitis B compared to those unvaccinated individuals.

2.8 Conceptual Framework

There are risk factors that expose blood donors to an increased risk of hepatitis B infection as shown in Figure 2. After being infected, there are a few possible outcomes which may vary from recovery after an acute infection or progression to chronic infection.



*MSM – men who have sex with men

Figure 2.2: Conceptual framework hepatitis B infection in blood donors

CHAPTER 3

MATERIAL AND METHODOLOGY

3.1 Introduction

Among the four transfusion-transmissible infections i.e. HIV, syphilis, HBV and HCV, hepatitis B infection remains the highest number of microbiological screening reactive among blood donors who had donated at NBC. A total of 1,736 donors which were inclusive of 575 first time donors were permanently deferred due to confirmed diagnosis of HBV infection between the year 2010 to 2015.

3.2 Study design

This is a cross-sectional study involving retrospective review of 215 blood donors' records who donated between 1st January 2010 and 31st December 2015 and were detected to be HBV positive at NBC.

3.3 Study venue and duration

The study was conducted at NBC, Kuala Lumpur from 28th June 2016 to 31st August 2017.

3.4 Study subject

The study subjects were selected via systematic random sampling after determining the potential study subjects according to the inclusion (Section 3.4.1) and exclusion (Section 3.4.2) criteria. The blood donors' records consisted of their donor enrolment form and clinic card. All the demographic data and laboratory results were recorded in the donors' proforma.

3.4.1 Inclusion criteria

First time Malaysian blood donors, that are screened positive for hepatitis B and donated at NBC from 1st January 2010 to 31st December 2015.

3.4.2 Exclusion criteria

i. Regular or lapsed donors.

ii. Donors who are not contactable.

iii. Donors who had donated blood at another centre prior to donation at NBC.

iv. Donors that did not turn up for investigation after notification letter sent out.

v. Donors that defaulted follow-up before confirmation of diagnosis for HBV infection.