Prevalence of Hepatitis B Virus DNA Among Blood Donors in Nablus- West Bank

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Adham Abu Taha

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DEDICATION

أيا صدى الروح ..
أهديك قافيتي.. وتراتيل اغترابي...
لعل أبعاد الكون.. تزاحم زوايا القلب..
لترسم فسحة سماوية.. وحلما لوطن لم يكتمل...
إلى كل من حبيت.. وأحب..
بعضا من بوحي....!
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The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: 

Signature: 

Date: 

Declaration
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<tr>
<td>HBV</td>
<td>Hepatitis B Virus</td>
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<tr>
<td>HBsAg</td>
<td>Hepatitis B surface Antigen</td>
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<td>HBeAg</td>
<td>Hepatitis B envelop Antigen</td>
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<td>Anti-HBc</td>
<td>Hepatitis B core Antibody</td>
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<tr>
<td>Anti-HBs</td>
<td>Hepatitis B surface Antibody</td>
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<td>HBV-DNA</td>
<td>Deoxyribonucleic acid of hepatitis B virus</td>
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<td>HBV cccDNA</td>
<td>Hepatitis B viral convalently-closed-circular DNA</td>
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<td>HCV</td>
<td>Hepatitis C Virus</td>
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<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<td>HCC</td>
<td>Hepatocellular Carcinoma</td>
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<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
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<td>OBI</td>
<td>Occult Hepatitis B Infection</td>
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<tr>
<td>NAT</td>
<td>Nucleic acid Amplification Test</td>
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<tr>
<td>NGHs</td>
<td>Non-Governmental Hospitals</td>
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<td>ORFs</td>
<td>Open Reading Frames</td>
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<tr>
<td>SAH</td>
<td>Specialized Arab hospital</td>
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<td>NSH</td>
<td>Nablus Specialty Hospital</td>
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<tr>
<td>MOH</td>
<td>Ministry of Health</td>
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<tr>
<td>ALT</td>
<td>Alanine Transaminase</td>
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<td>CHB</td>
<td>Chronic Hepatitis B</td>
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Prevalence of Hepatitis B Virus DNA among Blood Donors in Nablus- West Bank
By
Amira Majed Shiha
Supervisors
Dr. Adham AbuTaha
Dr. Kamal Dhumaidi

Abstract

Background: Detection of Hepatitis B Virus (HBV) DNA without Hepatitis B surface Antigen (HBsAg) with or without the presence of HBV seropositive antibodies outside the window period defines occult HBV infection (OBI).

Transmission of HBV from donors who were non-reactive for HBsAg and reactive for Hepatitis B core (anti-HBc) antibodies has been documented. Therefore the prevention of the residual risk of transfusion transmitted hepatitis B virus (HBV) could be minimized by testing blood, blood components, and organs of transplantation for antibodies against hepatitis B core antigen (anti-HBc) and or HBV-DNA.

Objectives: The objectives of the present study were to investigate the: 1) prevalence of anti-HBc antibodies among Palestinian blood donors, 2) prevalence of HBV-DNA among anti-HBc positive blood donors in Nablus blood bank centers.

Methods: Five hundred blood donors were selected by convenience sampling method from three blood donation centers in Nablus city. They were asked to fill out specially-designed questionnaires. Blood donors were
classified into two groups based on the motive for donation, therapeutic and non-therapeutic. Therapeutic blood donations were excluded and discarded. Specimens from blood donors were screened for HBsAg, anti HCV, and anti-HIV 1 and 2 at the collection sites. Blood units that passed the routine screening tests were tested for anti-HBc antibodies by ELISA. Samples that were positive for anti-HBc antibodies were tested for the presence HBV-DNA using home-made real time polymerase chain reaction (RT-PCR).

**Results:** A total of eleven (2.2%) donations were excluded from the study, 6 (1.2%) were therapeutic blood donations, 4(0.8%) were reactive to HBsAg, and 1(0.2%) was reactive to anti HCV. Forty nine (10%) of the remaining 489 blood specimens were reactive to anti-HBc antibodies. Forty five (92%) of them were positive for HBV DNA with an average of $13.5 \times 10^3$ copies/ml.

**Conclusion:** The prevalence of occult HBV among anti-HBc positive Palestinian blood donors was high. Therefore, introducing anti-HBc antibodies testing in the routine screening in blood bank centers will minimize the possibility of HBV transmission, taking into consideration the cost effectiveness of this test in comparison to the real time PCR.
Chapter One

Introduction and Literature Review
Chapter One
Introduction and Literature Review

1.1 Background

Hepatitis B virus (HBV) infection is a worldwide problem, two billion people have been infected with hepatitis B virus (HBV), 360 million have chronic infection, and 600,000 die each year from HBV-related liver disease or hepatocellular carcinoma (HCC) \[1\].

HBV can be transmitted vertically from mother to children or horizontally through sexual or household contact or by unsafe injections, but chronic infections acquired during infancy or childhood account for a disproportionately large share of worldwide morbidity and mortality. Vaccination against HBV infection can be started at birth and provides long-term protection against infection in more than 90% of healthy people \[1\]. Before 1970, approximately 6% of multi-transfused recipients acquired transfusion-transmitted HBV \[2\]. Over the last four decades, the risk of transfusion-transmitted hepatitis B virus has been steadily reduced, yet HBV transmission remains the most frequent transfusion-transmitted viral infection. The residual risk of HBV transfusion transmission is mainly related to blood donations negative for HBsAg that have been collected either during the pre-seroconversion ‘window period’ defined as the time between infection and detection of a viral antigen or antibody marker, or during the late stages of infection and with occult carriage of HBV infection (OBI) \[3\]. OBI is defined as the presence of HBV DNA without detectable HBsAg outside the window period. The critical question is
whether or not OBI is infectious by transfusion. All forms have been shown to be infectious in immunocompromised individuals, such as organ- or bone marrow-transplant recipients.

1.2 Hepatitis B Virus

Hepatitis B virus (HBV) belongs to the Hepadnavirida family. Humans are the only known natural host. HBV enters the liver via the bloodstream, and replication occurs only in liver tissue \[1\].

1.2.1 HBV Structure

The intact, infectious virus is 42–47 nm in diameter and circulates in the blood in concentrations as high as \(10^8\) virions per ml \[1\]. It is a circular double-stranded DNA virus, consisting of an inner nucleic acid, containing a core and an outer lipoprotein envelope, containing hepatitis B surface protein (also known as HBsAg). The virus secretes excess HBsAg into the serum of infected individuals, and this is the first serological marker to become detectable (Figure 1). It appears in the blood one to ten weeks after exposure and two to eight weeks before the onset of clinical hepatitis. Failure to clear HBsAg within six months indicates a chronic HBsAg carrier state \[4\].
Figure (1.1): Structure of the Hepatitis B virus as first described by Dane & alA simplified drawing of the HBV particle and surface antigen

The viral DNA is intimately associated with DNA polymerase, an enzyme which is important for the replication of the virus. Together they are surrounded by an inner coat of core protein, also known as HBCAg. HBCAg is found within the liver of infected individuals, but is insoluble and thus not detected in the blood. However the soluble component of HBCAg, called HBeAg, is secreted into the serum. HBeAg is cleared rapidly after acute infection but persists in the serum of patients with active viral replication.

1.2.2 HBV Genome

The genome of the HBV is a circular partially double stranded DNA molecule of about 3.2 kb in length that contains four overlapping open reading frames (ORFs) named S, P, C and X. The ORF S contains three initiation codons and encodes three polypeptides of different sizes called large, middle, and small that form the outer envelope of the virus pre-S1,
pre-S2, and S (hepatitis B surface antigen or HBsAg). The ORF P encodes the viral polymerase. The ORF C has two initiation codons that encode the hepatitis core antigen (HBcAg) that forms the nucleocapsid and a soluble antigen that is secreted into the blood stream, termed hepatitis B ‘e’ antigen (HBeAg), The function of the protein coded for by gene X is not fully understood (Figure 2).

![Figure (1.2): Organization of Hepatitis B virus genome. ORF stands for Open Reading Frame. The genes overlap. Created by :GrahamColm at en.wikipedia.](image)

### 1.2.3 Overview of the Hepadnaviral genome replication cycle

Replication of the hepadnaviral genome can broadly be divided into three phases (Figure 3): (1) Infectious virions contain in their inner icosahedral core the genome as a partially double-stranded, circular but not covalently closed DNA of about 3.2 kb in length (relaxed circular, or RC-DNA); (2) upon infection, the RC-DNA is converted, inside the host cell nucleus, into a plasmid-like covalently closed circular DNA (cccDNA); (3) from the cccDNA, several genomic and subgenomic RNAs are transcribed by cellular RNA polymerase II; of these, the pregenomic RNA (pgRNA)
is selectively packaged into progeny capsids and is reverse transcribed by the co-packaged P protein into new RC-DNA genomes. Matured RC-DNA containing-but not immature RNA containing-nucleocapsids can be used for intracellular cccDNA amplification, or be enveloped and released from the cell as progeny virions. Below we discuss these genome conversions, with emphasis on the reverse transcription step, and particularly its unique initiation mechanism [7].

Figure (1.3): Replication cycle of the hepadnaviral genome. Enveloped virions infect the cell, releasing RC-DNA containing nucleocapsids into the cytoplasm. RCDNA is transported to the nucleus, and repaired to form cccDNA (1). Transcription of cccDNA by RNA polymerase II (2) produces, amongst other transcripts (not shown), pgRNA. pgRNA is encapsidated, together with P protein, and reverse transcribed inside the nucleocapsid (3). (+)-DNA synthesis from the (-)-DNA template generates new RC-DNA. New cycles lead to intracellular cccDNA amplification; alternatively, the RC-DNA containing nucleocapsids are enveloped and released as virions. PM, plasma membrane [7].

1.2.4 Transmission

HBV is transmitted by percutaneous or mucosal exposure to infected blood or other body fluids. HBV transmission has been observed with numerous forms of human contact: perinatal/mother-to-child; household (nonsexual); sexual; needle-sharing; and occupational/health-care-related.
The highest concentrations of infectious HBV are found in blood and serum. However, other serum-derived body fluids, such as semen and saliva, are also infectious [8].

The risk of becoming a carrier is related to the age at the time of infection. The risk of chronic infection is highest for infants who acquire infection during the prenatal period (70 to 90%), lower for children younger than 5 years (20 to 50%) and lowest for older children and adults (5 to 10%) [9].

1.2.5 Clinical manifestation of Hepatitis B Virus infection

HBV infection may result in subclinical or asymptomatic infection, acute self-limited hepatitis, or fulminant hepatitis requiring liver transplantation. Persons infected with HBV may also develop chronic HBV infection, which can lead to cirrhosis or hepatocellular carcinoma. The likelihood that newly infected persons will develop chronic HBV infection is dependent on their age at the time of infection, more than 90 percent of infected infants, 25–50 percent of children infected between 1 and 5 years of age, and 6–10 percent of acutely infected older children and adults develop chronic infection [10].

Because of the inverse association between age and risk of chronic infection, persons infected as children assume a disproportionately large burden of morbidity and mortality attributable to HBV. Up to 25 percent of infants and older children who acquire HBV eventually develop HBV-
related hepatocellular carcinoma or cirrhosis. Adults who have had chronic HBV infection since childhood develop primary hepatocellular carcinoma at a rate of 5 percent per decade, which is 100–300 times the rate among uninfected persons \[11\].

**1.2.6 Global Patterns of transmission**

The global epidemiology of HBV infection has traditionally been described according to three categories of endemicity—high, intermediate, and low—depending on the proportion of the population that is seropositive for HBsAg (Figure 4). Countries with high endemicity are those where HBsAg seroprevalence is greater than or equal to 8 percent; countries with intermediate endemicity are those where seroprevalence is 2–7 percent; and those with low endemicity are those where seroprevalence is less than 2 percent \[12\]. HBsAg seroprevalence has marked geographic variations, and the degree of HBV endemicity often correlates with the predominant mode of transmission \[1\]. In highly endemic settings, perinatal and horizontal (exposure to chronically infected household members) routes are responsible for most disease transmission, and 70–90 percent of the adult population has serologic evidence of prior infection \[13\]. Because hepatocellular carcinoma HCC is a potential sequela of chronic HBV infection, highly endemic countries have markedly higher rates of liver cancer than countries with lower endemicity, and HCC is a major cause of mortality in these areas. Countries with intermediate endemicity have a mix of perinatal, horizontal, health-care-related, sexual, and other forms of
transmission. In countries with low endemicity, most new infections occur among young adults and are acquired sexually or through injecting drug use\textsuperscript{[1]}.

![Geographic prevalence of chronic hepatitis B](image)

**Figure (1.4): Geographic distribution of the prevalence of chronic hepatitis B virus infection\textsuperscript{[14]}**

### 1.3 Occult Hepatitis B Virus Infection

A silent form of hepatitis B infection called occult hepatitis B infection (OBI) has been recognized for nearly 20 years with the improvements in sensitivity of serological and genomic amplification assay, it is defined as the presence of HBV-DNA without detectable HBsAg with or without anti-HBc or anti-HBs, outside the pre-seroconversion window period. The frequency of occult hepatitis B infection depends on the relative sensitivity of both HBsAg and DNA assays, and also depends on the prevalence of HBV infection in the population. OBI may follow recovery from infection, displaying antibody to hepatitis B surface antigen (anti-HBs) and persistent low-level viremia, escape mutants undetected by the HBsAg assays, or healthy carriage with
antibodies to hepatitis B envelope antigen (anti-HBe) and to hepatitis B core antigen (anti-HBc) \cite{15}.

The risk of transfusion-transmitted HBV infection has been reduced by screening all blood donations for HBV surface antigen (HBsAg) since 1970\cite{16}, this measure was established in Palestine in 1988 in all blood banks. Although this serologic method reduced transfusion-transmitted HBV infections, some HBsAg-negative blood samples can still induce post transfusion hepatitis in recipients \cite{15}, which will be the silent source of infection, especially to the immunodeficiency patients. The frequency of post transfusion HBV infection is apparently due to the fact that HBsAg is circulating at very low and undetectable level for screening assays, nonetheless, anti-HBc screening tests are able to eliminate some of these donor units\cite{17}, whereas anti-HBc Abs is a marker of acute, chronic or resolved HBV infection and remains detectable for life. These can be present in the absence of both HBsAg and anti-HBs Abs, during the convalescent period following acute hepatitis B before the appearance of anti-HBs Abs, or in patients who resolved infection but lost detectable anti-HBs Abs, anti-HBc is therefore detected in anyone who has been infected with HBV \cite{17}.

1.3.1 Definition

The presence of HBV DNA in the liver (with detectable or undetectable HBV DNA in the serum) of individuals testing HBsAg negative by currently available assays. When detectable, the amount of HBV DNA in the serum is usually very low (<200 IU/ml) \cite{18}.
As a matter of fact, the molecular basis of occult HBV infection has been related to the long-term (life-long) persistence in the nuclei of the hepatocytes of the viral covalently-closed-circular DNA (HBV cccDNA).

On the basis of the HBV antibody profile, OBI may be distinguished as: Seropositive-OBI (anti-HBc and/or anti-HBs positive) and seronegative-OBI (anti-HBc and anti-HBs negative). In seropositive-OBI subjects, serum HBsAg may become negative either following the resolution of acute hepatitis B (thus, after a few months of HBsAg carriage) or after years of chronic HBsAg positive infection \[^{[19]}\] (Fig. 5). The seronegative-OBI cases might have either progressively lost the hepatitis B specific antibodies or theoretically, the individual may have been hepatitis B specific antibody negative from the beginning of the infection (Fig. 5).

_“False” OBI:_ Cases with serum HBV DNA levels comparable to those usually detected in the different phases of serologically evident (overt) HBV infection have to be considered as “false” OBI and are usually due to infection by HBV variants with mutations in the S gene (escape mutants) producing a modified HBsAg that is not recognized by some or all commercially available detection assays \[^{[20]}\].
1.3.2 Mechanisms leading to occult HBV infection

Four main mechanisms may lead to occult HBV infection. Three of them are operating in chronically infected individuals:\textsuperscript{[15]}

- Undetectable HBsAg at the tail end of chronic carriage during the long term non-replicative phase following anti-HBe conversion.

- The occurrence of escape mutants interfering with HBsAg (false OBI) synthesis or detectability by various serological assays. Envelope mutant forms of hepatitis B virus (HBV), impairing HBV antibody recognition, have been reported with mutations in single or multiple sites of the hepatitis B surface antigen (HBsAg) group-specific "a" determinant. Blood donors infected with such an HBsAg mutant form of HBV may escape detection by HBsAg screening assays and therefore may affect the safety of the blood supply\textsuperscript{[21]}. 

\textbf{Figure (1. 5): Schematic representation of HBV profile in OBI and “false” OBI \[18\].}
• The interference of other viruses in HBV replication, primarily the defective Delta virus, but possibly others.

• The last type of situation occurs in individuals who have recovered from an acute infection in whom HBV persists mostly in sanctuaries such as the liver tissue but also occasionally in the circulation.

1.3.3 Epidemiology of Occult Hepatitis B virus

Occult HBV infection is world-wide spread, although its distribution might reflect the general prevalence of HBV in various geographic areas and among various populations \[22\]. The epidemiology of OBI depends on the mode of transmission of HBV infection, which is critical as vertical transmission and horizontal transmission before age five results in frequent chronic infections which, after varying periods of time according to the genotype, reach the non-replicative stage that leads to occult infection. In contrast, in areas where the epidemiology is dominated by transmission by sexual or intravenous drug use, in adults, most infections will recover and occult infection will be found in persistent recovered cases \[15\].

HCV infected patients appear to be a category of individuals with higher prevalence of occult HBV \[22\]. In particular, HBV-DNA is detectable in about one-third of HBsAg-negative HCV carriers in the Mediterranean basin, and this prevalence is even higher in Far East Asian countries \[23\]. Prevalence of OBI appears to be fairly elevated even in patients with cryptogenic liver disease, particularly in those with cirrhosis. Again, the
available data provide incomplete information. Among blood donors, this cryptic infection appears to be a rare occurrence in the western world, whereas it is frequently detected in the developing countries \cite{22}.

1.3.4 Diagnosis of occult HBV infection

The diagnosis of occult HBV relies on the differential sensitivity between HBsAg and HBV DNA assays. All the experts in the field are in agreement that they should consider HBV-DNA the only reliable diagnostic marker of OBI. Only if highly sensitive HBV-DNA testing is not feasible, anti-HBc should be used as a less than ideal surrogate marker in order to identify potential seropositive OBI individuals in cases of blood, tissue or organ donation and when immune suppressive therapy has to be administered \cite{18}. Since OBI is characterized by a viral load in circulation generally below (2x10E3 IU/ml) or (1x10E4 copies/ml), it is strongly recommended to utilize a highly sensitive and specific approach based on “nested” or “real time” PCR techniques and the use of oligonucleotide primers specific for different HBV genomic regions and complementary to highly conserved (genotype shared) nucleotide sequences \cite{18}.

As in all genomic amplification methods, taking draconian precaution to avoid contamination is even more critical when attempting to identify very low levels of viral genome.

1.3.5 Clinical impact

OBI may impact in several different clinical contexts, including the possible transmission of the infection, the risk of reactivation, the
contribution to liver disease progression and to the development of hepatocellular carcinoma (HCC). OBI status is mainly related to a strong suppression of the viral activity in which the host’s immune surveillance is likely to play a major role. However, this state of suppression of viral replication and gene expression may be discontinued, leading to the development of a typical hepatitis B that often has a severe – and sometimes even fulminant – clinical course. This event is usually observed in patients under condition of immunosuppression induced by therapies and/or related to diseases that involve the immune system [24]. As a matter of fact, an occult HBV carrier when becoming immunocompromised may show a reactivation of the viral replication because of the fault of the immunological control. Studies performed in different geographic areas have shown a significant association between OBI and severe liver diseases particularly in patients with HCV infection, thus suggesting that occult HBV may favor or accelerate the progression towards cirrhosis in these individuals [25]. Moreover, several reports indicate that occult HBV infection is associated with the progression of liver fibrosis also in patients with cryptogenic liver disease [26]. Finally, much evidence indicates that occult HBV infection is a risk factor for HCC development [22].

1.4 Significance of this study

Organ transplant, cancer, hemodialysis, thalassiemia, and hemophilia patients are at higher risk of developing OBI, since these patients are immunocompromised and need frequent blood transfusions.
These patients should receive HBV free blood units. HBV-DNA testing is not a routine test in blood banks and it would be very expensive to screen all blood units for HBV-DNA.

One way to minimize the occurrence of OBI is to screen HBsAg-neg. blood for anti-HBc and test these patients for the presence of HBV-DNA.

1.5 Objectives of the study

Main objectives

A. To determine the prevalence of anti-HBc Abs in blood units that pass routine screening tests in blood banks.

B. To determine the prevalence of HBV-DNA in these units (blood units that pass routine screening tests in blood banks).

1.6 Literature review

After the introduction of reliable serologic screening of blood donations, post transfusion hepatitis has become rare. However, the identification of blood donors with occult HBV infection (donors who are negative for HBsAg but have detectable circulating HBV DNA) has created some concern with regards to the safety of blood supply. It is generally accepted that the diagnosis of infection by HBV is based on the presence of the HBsAg in the bloodstream. However, screening of blood bank donors for HBsAg does not totally eliminate the risk of HBV
infection through blood transfusion, since the absence of this marker in the serum does not exclude the presence of HBV DNA. It is possible that, donors with occult HBV infection, who lacked detectable HBsAg but whose exposure to HBV infection was indicated by a positive anti-HBc and HBV DNA, are a potential source of HBV infection [27]. Mosley et al suggested that anti-HBc screening of blood donations might prevent HBV transmission from HBsAg-negative blood donors that are positive for anti-HBc [28]. The prevalence of OHB varies significantly between geographical regions as well as among various patient populations tested. It also depends upon the assay employed in routine serological or nucleic acid test (NAT) screening. Occult HBV infection has been reported in 0.1–2.4% of HBsAg-negative, anti-HBc–positive (±anti-HBs) blood donors in Western countries such as the United States where only 5% of the population has prior exposure to HBV and in up to 6% of a similar cohort of donors who reside in endemic areas where 70–90% of the population has been exposed to HBV. Correspondingly, among the general population in Asia with normal ALT levels, the prevalence of OHB has ranged from 7.5% to 16% [29]. The prevalence of anti-HBc only in Europe and North America is overall quite low. Anti-HBc positive prevalence rates among HBsAg-negative blood donors that vary from 0.56% in the United Kingdom [30], 0.84% in United States [31], 1.4% in Germany [16], 15.03% in Greece [32], 1.13% in Canada [33], less than 2% in Switzerland [16], in Italian blood donors anti-HBc positive was 4.85% [34]. In areas of higher HBV infection prevalence about 20%-70% of subjects are positive for anti-HBc antibody, many of studies were
conducted in Iran and the prevalence of anti-HBe positive varied from 5.18 to 8% [35]. While in India 18.9%, and in Pakistan 17.28% [27], also 20% in Turkey [36] to 76% in Ghana [37]. On the other hand some Arab countries has reported the prevalence of anti-HBe positive such as 17.4% in Saudi Arabia [38], Oman 20.5 % [39], Egypt 11.54% [40], and 3.7% in Lebanon [41]. Moreover, the percentage of samples containing HBV-DNA from either blood donors or the general population ranges between 0% to 7.7% in areas where the HBV prevalence is low, and in areas of higher HBV infection prevalence, such as Ghana, the frequency of DNA positive anti-HBe alone cases increases to 12.7%, these discrepancies in the rate of occult HBV infection may reflect the diverse prevalence of HBV infection in different countries, the sensitivity of the various molecular biology techniques used, and the size and virological features of the patient groups [42]. the incidence of HBV-DNA in only anti-HBe positive blood donors has been reported to be 0% in brazil [43], 0.3% in china [44], 1.1 % in Japan [45], 12.7% in Ghana [37], in Taiwan 0.11% [46], 1.25% in Saudi Arabia [47], 1.26% in Egypt [40], and 5.4% in Lebanon [41]. In high prevalence areas, HBV-DNA detected by PCR is found in 4-24% of the population in India [27], Taiwan [46], Japan [45], and Sardinia [48], in West Africa, approximately 5% of total HBV-DNA carriers are HBsAg negative [49]. Palestine is considered according WHO classification, country with intermediate endemicity for HBV [12]. A survey conducted by Dr. As’ad Ramlawi with preventive medicine team (Ministry of Health MOH) in 2000 shows a prevalence of 3.4% among all the population over 8 years old [6]. Since 1992, the vaccination for HBV was
implemented for newborn in national Expanded Program for Immunization (EPI) and is available for population at high risk of HBV infection but didn't include neither children nor young adults 'catch-up vaccination'. In Palestine, screening of blood donors for HBsAg became obligatory since 1988 but routine testing for anti-HBc of blood units has never been implemented. Actually the frequency of HBsAg positive among blood donors in blood bank centers in Palestine is 1.7 % (MOH) \(^{[50]}\).
Chapter Two
Methodology
Chapter Two  
Methodology

2.1. Type of study

A Cross-sectional study was conducted to investigate the prevalence of anti-HBc-antibodies and the occult HBV among blood donors in Nablus district. The study was approved by the ethics committee at An-Najah National University.

2.2. Place of study

City of Nablus /Palestine

2.3. Setting

Three blood bank centers; one governmental (Rafidia Surgical Hospital), and two private hospital blood banks (Specialized Arab Hospital, Nablus Specialty Hospital)

2.4. Study design and population

The study population was participants who came to the blood bank centers to donate their blood. The average number of blood donors in the Nablus District is 8572/year. The study sample was calculated using the following equation:

Sample size \( (SS) = z^2 \times (1-P)/d^2 \)

Where: \( Z \): statistic for a level of confidence,
\textbf{P}: is the estimated prevalence rate, and \textbf{d}: precision, the confidence level 95\% and percentage of error 2\% \textsuperscript{[5]}\textsuperscript{1}. The estimated sample size was 370 participants.

A convenience sampling method was used to select 500 blood donors during the period of February to August 2009.

All participants were asked to fill out blood donation questionnaires. Based on the motive of donation, donors were classified into two groups: therapeutic and non-therapeutic.

Blood specimen was taking from each donor to investigate the presence of Anti Hbc antibodies and HBV DNA. These blood specimens were aliquoted into two eppendorf tubes and kept at -20 \textdegree C for further testing.

\textbf{2.5 Inclusion criteria, Exclusion criteria}

\textbf{Inclusion criteria}

Non-therapeutic blood donations, which tested negative for HBsAg, HCV, and HIV 1 & 2.

\textbf{Exclusion criteria}

1- Therapeutic blood donations.

2- Non therapeutic blood donations which tested positive for HbsAg and/or anti HCV and/or Anti HIV1& 2
2.6 Laboratory methods

All blood specimens were initially screened at the blood bank centers, for HbsAg, Anti-HCV and Anti- HIV 1 & 2 using ELISA assay (AUSAB-Abbott/USA) according to the manufacturer's instructions, (applied Axsym-Abbott/USA).

Blood units that yield negative results for all the three initial screening tests were tested in duplicates for Anti-HBc antibodies at the Central Public Health Lab, Ramallah using ELISA assays (manufactured by:Dade Behring,Marburg/Germany) according to the manufacturer's instructions using(microplate ELISA Washer: Stat Fax 2600 manufactured by: Awareness Technology/USA, and microplate ELISA Reader: METERTECH manufactured by: Metertech Taiwan or USA).

All positive samples for anti-HBc-IgG were tested for the presence of HBV-DNA at the Medical Research Center in Al-Quds University using real-time polymerase chain reaction (RT-PCR) technique.

For this, one hundred µl sample's serum was subjected to DNA extraction using QIAampDNA mini kit (cat no.51304, Qiagene, Germany) as follows:

1. 20 µl Qiagen Proteinase K were pipetted into the bottom of a 1.5 ml microcentrifuge tube.

2. 200 µl samples were added to the microcentrifuge tube. If the sample volume is less than 200 µl, PBS was added to adjust the amount.
3. 200 μl Buffer AL were added to the sample, mixed by pulse-vortexing for 15 s.

4. Mixture was incubated at 56ºC for 10 min followed by a short spin down.

5. 200 μl ethanol (96-100 %) were added to the sample, mixed by vortexing and briefly centrifuged to remove drops from the inside of the tube lid.

6. Mixture from step 5 was applied carefully to the QIAamp Mini spin column (in a 2ml collection tube) without wetting the rim. The cap was closed and column in the collection tube was centrifuged at 8000 rpm for 1 min. Place the QIAamp Mini spin column in a clean 2ml collection tube, and discard the tube containing the filtrate.

7. 500 μl Buffer AW1 were added to the QIAamp Mini spin column and add without wetting the rim and centrifuged at 8000 rpm for 1 min. Filtrate was discarded.

8. The QIAamp Mini spin column was replaced in a clean 2 ml collection tube, 500 μl Buffer AW2 was added and. Filtrate was discarded and centrifuging step was repeated.

9. Finally, the QIAamp Mini spin column was placed in a clean microcentrifuge tube, 200 μl elution buffers was added to the column and centrifuged at 14000 rpm for 3 min. The filtrate in this step resembles the isolated DNA.
Real-time PCR was performed using an ABI Real Time PCR 7500 (applied biosystems, USA). All positive controls, negative controls and samples were tested in duplicates. A total of 25-µl reaction mixture consisted of 5µl control (positive control; standard, negative control, ultra pure water) or sample's DNA, 12.5 µl TaqMan universal master mix (Applied Biosystems), 1 µl of each primer (forward and reverse, each at 10 pmol/µl), 0.4 µl probe (100 nmol/µl) and 5.1 µl ultra pure water.

The amplification reaction started with 2 min at 50°C, followed by 10 min at 95°C and final 45 cycles as following: 95°C for 15s and 60°C for 1 min. The primer pair (PF: 5'-GAC GTC CTT TGT YTA CGT CGG GTC- 3', PR: 5'- TGC AGA GGT GAA GCG AAG TGG CCA- 3' and probe (FAM 5'- ACG GGG CGC ACG TCT CTT TAC CCG G-3' – MGBNFQ) were specific to the X gene of the HBV virus. (Note: PCR methods were optimized at the medical research center in Al-Quds University). Validated HBV-DNA complete genome (Clonit, Italy) at 10^6 serially diluted in ultra pure water down to 10^1 copies/µl was utilized as standard in all RT-PCR assays. To rule out the possibility for carry over between samples, four were subjected to sequencing analysis. For this PCR product of six different HBV positive samples were purified employing MinElute PCR purification Kit (Cat. No. 28004, Qiagen, Germany). Sequencing was performed at the heredity lab of the Bethlehem University using sequencer machine ABI PRISM 3130 Genetic Analyzer. The sequencing PCR reaction was performed with the forward primer in each reaction and BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied
Biosystems, USA, cat no. 4337451-100). Sequences were analyzed using the DNASTar program (DNASTAR Inc., Madison, WI, USA). Sequences were aligned using the Clustal W methods against each other and HBV x gene sequence downloaded from the NCBI (accession no.325515647). Using the DNASTar program, a phylogenetic tree (Fig.2.1) showing the relevance of the different sequences to each other was established using the percent identity and divergence of the sequences calculated by the program. Finally, the confirmation of this analysis was ensured by adding the calculated bootstrapping value to the phylogenetic tree (Fig.2.2).

![Nucleotide Substitution per 100 residues](image)

**Fig. (2.1): phylogenetic tree**
2.7 **Statistical analysis**

The Statistical Package of Social Sciences (SPSS) version 15 was used in statistical analysis. Cross tabulation, Chi square ($X^2$), and ANOVA test were used to detect the significant differences between the various groups, *P*-value less than 0.05 was considered significant.

2.8 **Ethical issues**

Permissions were obtained from the Palestinian Ministry of health, and the administrations of both SAH and NSH to conduct this study in the chosen blood banks.

A signed consent form was obtained from all blood donors.
Figure (2.2): Flow chart explaining the laboratory methods

Blood donors

Therapeutic

Discard

Non-Therapeutic

Screening tests HBsAg, anti-HCV, anti-HIV1/2

Negative

Anti-HBc ELISA

Positive

Discard

Negative

HBV-DNA RT-PCR

Positive

Discard

Negative
Chapter Three

Results
Chapter Three

Result

3: General characteristics of study sample

A total of 500 blood donations were selected from both the governmental and private blood banks (390, 110 respectively) in Nablus district. The mean age of donors is 34.02 ±9.59 years (range: 18 – 58).

Four hundred and eighty seven (99.6%) blood donations came from males and 2 (0.4%) from females. Three hundred and twenty six (67.6%) donations were collected from volunteers, 156 (32.4%) units were donated by family members.

Four hundred and twenty nine (89%) out of the 489 respondents were born in Palestine, and 53 (11%) were born in others countries, "KSA and Kuwait (3.1%), Jordan (1.8%), Syria, Libya (0.4%), 0.2% for Iraq, Algeria, UAE, Dubai, Abu-Dhabi, and Venezuela".

3.1 Serological screening of blood donations

A total of 5 (1%) blood donations were rejected from the study where 4 (0.81%) units were positive for HBsAg and 1 (0.2%) donation tested positive for HCV antibodies.

Therefore, 489 (487 male and 2 female) blood donations passed the routine screening tests and were eligible for transfusion. These units were tested for the presence of anti-HBe (Fig.3.1).
Forty nine blood units were positive for anti-HBc antibodies with a prevalence rate of 10%. Table 3.1 shows the distribution of anti-HBc antibodies among donor age groups, where was the highest age group (20-29 years, 32.7%), and more than two third of units was < 40 years.
Table (3.1): Distribution of anti-HBc positivity by age group of donors.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 20 yr.</td>
<td>2</td>
<td>4.1%</td>
<td>4.1%</td>
</tr>
<tr>
<td>20-29 yr.</td>
<td>16</td>
<td>32.7%</td>
<td>36.8%</td>
</tr>
<tr>
<td>30-39 yr.</td>
<td>15</td>
<td>30.6%</td>
<td>67.4%</td>
</tr>
<tr>
<td>40-49 yr.</td>
<td>15</td>
<td>30.6%</td>
<td>98%</td>
</tr>
<tr>
<td>More than 50 yr.</td>
<td>1</td>
<td>2%</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

3.2 Anti-HBc positivity and personal information variables

The number of donors who are positive for anti-HBc antibodies and less than 40 years old was 33; while 16 donors where older than 40 years and positive for anti-HBc antibodies (table: 3.2).

All 49 anti-HBc positive units were from male donors who were born in Palestine.
Concerning the residence of blood donors, the rate was found to be significantly different (p<0.05) between the three places of residence, however the prevalence rate of the anti-HBe positive was found to be higher in refugee camps (9/44:20.5%) than rural (24/257:9.3%) and urban areas (16/188:8.5%).
In (table.3.2) there was not a significant difference between anti-HBc positivity and job, educational level, and monthly income. There was no significant difference in the positivity of anti-HBC antibodies and the job status (table.3.2) where 36 (11.1%) donors had jobs and 4 (10.8%) was jobless.

On the other hand, data analysis of educational levels indicated that the highest prevalence was among the illiterates followed by people with primary level and university graduate. The lowest incidence was among people of secondary school level \{(13/117:11.1%), (15/138:10.9), (19/207:9.2%), 2/14:14.3\}% respectively.

There was no statistical difference between anti-HBc positivity and income; 42 (10.6%) people had a low income (less than 3000 NIS) and 5 people (11.4%) with more than 3000 NIS.

However, the prevalence rate of anti-HBc positive was found to be significantly different by family status (P<0.05), whereas the prevalence was higher in married (37/246:15%) than in single (12/233:5.2%).

With regard to the motive for blood donation, there was no statistical difference among the three reasons for blood donations and the positivity of anti-HBc antibodies as shown.

3.3 Anti-HBc positive and medical history variables:

Table 3.3 represents the relationship of anti-HBc positive with the medical history variables.
### Table (3.3): Relationship of Anti-HBcIgG positivity with medical history of donors.

<table>
<thead>
<tr>
<th></th>
<th>Anti-HBcIgG Result</th>
<th>Total</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td><strong>Hepatitis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14(87.5%)</td>
<td>2(12.5%)</td>
<td>16</td>
</tr>
<tr>
<td>No</td>
<td>359(90%)</td>
<td>40(10%)</td>
<td>399</td>
</tr>
<tr>
<td>Unknown</td>
<td>56(88.9%)</td>
<td>7(11.1%)</td>
<td>63</td>
</tr>
<tr>
<td><strong>chronic diseases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>24(82.8%)</td>
<td>5(17.2%)</td>
<td>29</td>
</tr>
<tr>
<td>No</td>
<td>405(90.2%)</td>
<td>44(9.8%)</td>
<td>449</td>
</tr>
<tr>
<td><strong>Previous surgery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>128(92.1%)</td>
<td>11(7.9%)</td>
<td>139</td>
</tr>
<tr>
<td>No</td>
<td>303(88.9%)</td>
<td>38(11.1%)</td>
<td>341</td>
</tr>
<tr>
<td><strong>I.V Use</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8(80%)</td>
<td>2(20%)</td>
<td>10</td>
</tr>
<tr>
<td>No</td>
<td>418(89.9%)</td>
<td>47(10.1%)</td>
<td>465</td>
</tr>
<tr>
<td><strong>Donation No.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First time donor</td>
<td>123(89.9%)</td>
<td>14(10.2%)</td>
<td>137</td>
</tr>
<tr>
<td>Not first time</td>
<td>309(90.1%)</td>
<td>34(9.9%)</td>
<td>343</td>
</tr>
<tr>
<td><strong>Vaccinated against HBV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>23(92%)</td>
<td>2(8%)</td>
<td>25</td>
</tr>
<tr>
<td>No</td>
<td>387(89.4%)</td>
<td>46(10.6%)</td>
<td>433</td>
</tr>
<tr>
<td>Don't know</td>
<td>21(95.5%)</td>
<td>1(4.5%)</td>
<td>22</td>
</tr>
<tr>
<td><strong>Previous dental work</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>264(88.6%)</td>
<td>34(11.4%)</td>
<td>298</td>
</tr>
<tr>
<td>No</td>
<td>167(91.8%)</td>
<td>15(8.2%)</td>
<td>182</td>
</tr>
<tr>
<td><strong>History of needle puncture</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>33(91.7%)</td>
<td>3(8.3%)</td>
<td>36</td>
</tr>
<tr>
<td>No</td>
<td>395(89.6%)</td>
<td>46(10.4%)</td>
<td>441</td>
</tr>
<tr>
<td>Don't know</td>
<td>2(100%)</td>
<td>0(0%)</td>
<td>2</td>
</tr>
<tr>
<td><strong>blood received previously</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11(84.6%)</td>
<td>2(15.4%)</td>
<td>13</td>
</tr>
<tr>
<td>No</td>
<td>418(89.9%)</td>
<td>47(10.1%)</td>
<td>465</td>
</tr>
<tr>
<td>Don't know</td>
<td>2(100%)</td>
<td>0(0%)</td>
<td>2</td>
</tr>
</tbody>
</table>

In table 3.3 There was no statistical difference between the positivity of anti-HBc positivity and the being a first-time donor or not.

As regards to vaccinated against HBV, the table 3.3 shows not found to be significantly different (P>0.05) between blood donors vaccinated against HBV or not, although the rate of blood donors not vaccinated was
highly than those vaccinated or don't know respectively (46/433:10.4%, 2/25:8%. 1/22:4.5%).

Moreover, the prevalence rate of anti-HBc positive among donors with previous dental work and history of needle puncture showed no significant difference between who exposed to the dental work and needle puncture or not, nevertheless the donors with history of previous dental work showed higher prevalence (33/297:11.1%), contrarily the donors not exposed to needle puncture showed higher prevalence (45/439:10.3%).

3.4 Anti-HBc positive and behavioral risk factors

The relationship of anti-HBc positive with various variables of behavioral risk factors was illustrated in table 3.4. A significant difference (P<0.05) on the prevalence rate of anti-HBc positive among tattoo donors used or not used was demonstrated. The prevalence rate was very high of those who used tattoo(11/47:23.4%), while the rate of those who didn't use tattoo was low vis-à-vis the precedent result (38/433:8.8%). The results showed that there was a significant difference (P<0.05) on the prevalence of anti-HBc positive among donors with household contacts of HBV carriers (7/19:36.8%) and those without (34/407:8.4%), whereas those who reported as don't known in questioner was (8/50:16%).

The other behavioral risk factors (sharing shaving blades, living with patient who received blood, and living with immunosupprition) appear no significant.
Table (3.4): Relationship of Anti-HBc IgG positivity with behavioral risk factors of donors.

<table>
<thead>
<tr>
<th></th>
<th>Anti-HBc IgG Result</th>
<th>Total</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Sharing shaving blades</td>
<td>Yes</td>
<td>69(93.2%)</td>
<td>5(6.8%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>291(89.3%)</td>
<td>35(10.7%)</td>
</tr>
<tr>
<td></td>
<td>At the barber</td>
<td>71(88.8%)</td>
<td>9(11.3%)</td>
</tr>
<tr>
<td>tattoo</td>
<td>yes</td>
<td>36(76.6%)</td>
<td>11(23.4%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>395(91.2%)</td>
<td>38(8.8%)</td>
</tr>
<tr>
<td>Living with a patient who received blood</td>
<td>Yes</td>
<td>13(86.7%)</td>
<td>2(13.3%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>418(89.9%)</td>
<td>47(10.1%)</td>
</tr>
<tr>
<td>Living with immunosuppressed patient</td>
<td>Yes</td>
<td>23(85.2%)</td>
<td>4(14.8%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>407(90%)</td>
<td>45(10%)</td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td>Yes</td>
<td>27(87.1%)</td>
<td>4(12.9%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>403(90%)</td>
<td>45(10%)</td>
</tr>
<tr>
<td>Partner of HBV carrier</td>
<td>No</td>
<td>174(86.1%)</td>
<td>28(13.9%)</td>
</tr>
<tr>
<td></td>
<td>Don't know</td>
<td>37(80.4%)</td>
<td>9(19.6%)</td>
</tr>
<tr>
<td>Household contact of HBV carrier</td>
<td>Yes</td>
<td>12(63.2%)</td>
<td>7(36.8%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>373(91.6%)</td>
<td>34(8.4%)</td>
</tr>
<tr>
<td></td>
<td>Don't know</td>
<td>42(84%)</td>
<td>8(16%)</td>
</tr>
</tbody>
</table>

3.5. Frequency of HBV DNA among anti-HBc positive donations

Forty five (91.8%) of the anti-HBc positive donations were HBV-DNA positive by Real- Time PCR. The result showed that the viral load was between \(1 \times 10^2 - 6.1 \times 10^5\) \{average 13.5 \times 10^3 copies/ml\}, the mean age of donors with HBV DNA was 33.04± 9.09 (range: 18-49).

The frequency of HBV-DNA positive by age was found to be significantly different (P<0.05) between age groups, the frequency rate was
however higher in the age group between 20-39 year 28/45(62.2%), whereas 13/45(28.9%) of donations was between 40-49 year and 4/45 (8.9%) less than 20 year (table 3.5).

Table (3.5): Distribution of HBV-DNA by age group

<table>
<thead>
<tr>
<th>Age group</th>
<th>HBV-DNA by PCR</th>
<th>Total</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>-ve</td>
<td></td>
</tr>
<tr>
<td>Less than 20 year</td>
<td>4(100%)</td>
<td>0(0%)</td>
<td>4(8.2%)</td>
</tr>
<tr>
<td>20-29 year</td>
<td>14(100%)</td>
<td>0(0%)</td>
<td>14(28.6%)</td>
</tr>
<tr>
<td>30-39 year</td>
<td>14(93.3%)</td>
<td>1(6.7%)</td>
<td>15(30.6%)</td>
</tr>
<tr>
<td>40-49 year</td>
<td>13(86.7%)</td>
<td>2(13.3%)</td>
<td>15(30.6%)</td>
</tr>
<tr>
<td>More than 50</td>
<td>0(0%)</td>
<td>1(100%)</td>
<td>1(2%)</td>
</tr>
<tr>
<td>Total</td>
<td>43(87.8%)</td>
<td>6(12.2%)</td>
<td>49(100%)</td>
</tr>
</tbody>
</table>
Chapter Four

Discussion and Recommendations
4.1 Discussion

The risk of infection with transfusion–transmitted viruses has been reduced remarkably since the introduction of serological screening. However, a zero-risk blood supply remains a goal. The risk of transfusion–transmitted HBV infection has been reduced by screening all blood donations for HBsAg since 1970[15], it was generally accepted that the disappearance of HBsAg indicates the clearance of HBV. Meanwhile, many reports pointed to occurrence of post-transfusion hepatitis B [40]. At present, HBsAg detection is the only diagnostic screening test for HBV infection in blood transfusion centers in Palestine since 1988. In this study the main objectives was to determine the prevalence of anti-HBc Abs in blood units that pass routine screening tests in blood banks, and to determine the prevalence of HBV-DNA in these units (blood units that pass routine screening tests in blood banks).

We examined 494 samples obtained from healthy blood donors and found that the prevalence of HBsAg was 0.8% with mean age (27.5 ± 8.06), while much lower from the prevalence reported in an earlier national study which was conducted in the year 2000 among Palestinian students (3.4%) [6].

This variation could be related to the small sample size (494 samples vs. 2000 samples) first, and the location of study (Nablus vs. West Bank &
Gaza strip) second, and the age group selected (adult vs. school age). The prevalence of HCV was 0.2% similar to the result of the previous study (0.3%). Compared to the neighboring Arab countries; it was similar to Lebanese blood donors (0.9%) \(^{[41]}\), while it was higher in Egypt, Oman, and Saudi Arab (1.18%, 2.8%, 3.6%) respectively \(^{[40, 39, 38]}\).

### 4.1.1 The prevalence of anti-HBc among blood donors

This study showed that out of the 489 blood screened donors, 49 (10%) were found to be ‘anti-HBc alone’ positive which is almost twelve times higher than the HBsAg positivity rate (0.8%), this high prevalence is similar to that in Egypt (10.96%) \(^{[40]}\) and Saudi Arabia (17.4%) \(^{[38]}\), in contrast to a lower prevalence of (3.7%) in Lebanese blood donors \(^{[41]}\), however the highest prevalence of anti-HBc was reported among Omani blood donors (20.5%) \(^{[39]}\). In Iran, Behzad-Beibahani et al (2005) have found that 6% of Iranian blood donors' samples were negative for HBsAg but positive for both Anti-HBc and anti-HBs \(^{[52]}\). In contrast, another study has found that blood components containing anti-HBc and anti-HBs, even at low level; do not appear to transmit HBV \(^{[28]}\).

### 4.1.2 Anti-HBc positivity and demographic variables among blood donors

On the other hand, the highest prevalence of anti-HBc was reported in the age group of 30-39 years (31.3%), this may be related to high prevalence of HBV infection among people who were born before 1980,
while there was no screening to blood units, or to vertical transmission and horizontal transmission before age five results in frequent chronic infections which leads to occult infection, the lowest prevalence of anti-HBe was among age group more than 50 years (2%), the same result reported among this age group in Egypt (30-41 years "27.69%) which related to national campaign of parenteral schistosomal treatment implemented since the 1960s and ended in the mid-1980s of past century. The lowest prevalence of anti-HBe was among the age group of 18–20 years (7.32%; \( P = 0.02 \)), which may represent the generation born after stoppage of parenteral antibilharzial treatment \[40\]. With regard to residence, no studies before indicated to this variable, this study was significant differences in the residence, whereas more apparent in camps, which are too crowded, and the transmission of HBV more easily.

4.1.3 Anti-HBe positivity and its medical and behavioral history variables among blood donors

In the medical history, there were significant differences (\( p \) value less than 0.05) in the family history of CHB 36.8% (7/19), 16% (8/50) was not sure, and 8.4% (34/373) had no family history, this result correspond to the above result, where was correlation between anti-HBe positive on one hand and the blood donations living in camps on the other hand, one study indicated to this point but in contrast it had no significant differences in contact with householder previous infected to HBV\[53\]. Also, no study
previously was discuss tattoo use, where was a clear relationship with positive blood donations to anti-HBc.

4.1.4 anti-HBc positivity and its HBV-DNA among blood donors

It is noteworthy that in areas with low HBV prevalence, not more than 5% of HBsAg (−)/anti-HBcAb (+) blood units contains HBV-DNA. In contrast, in high prevalence areas (such as India and Taiwan), serum HBV-DNA is found in 4%–25% of the HBsAg (−) and anti-HBc (+) population\textsuperscript{[54]}. The prevalence of OBI is quite variable depending on the level of endemic disease in different parts of the world, the different assays utilized in the studies, and the different populations studied\textsuperscript{[55]}. The studies of prevalence in North America reveal that HBV DNA was detected in 0.1%-1.05% of those who were HBsAg negative and anti-HBc-positive (with or without anti-HBs) and that HBV DNA was detected in 2.03%-2.8% in the anti-HBc only category (no anti-HBs)\textsuperscript{[56,57]}. The studies of prevalence in Europe reveals that HBV DNA was detected in 0%-1.59% of those who were HBsAg negative and anti-HBc-positive (with or without anti-HBs) and HBV DNA was not detected in patients who were anti-HBc only\textsuperscript{16,58}. The studies of prevalence in the Middle East and Asia revealed that HBV DNA was detected in 1.09%-3% of those who were HBsAg negative and anti-HBc-positive (with or without anti-HBs) and that HBV DNA was detected in 8.1% in the anti-HBc only category (no anti-HBs)\textsuperscript{[55]}. 
In our study the overall prevalence of occult HBV infection in healthy blood donors was 91.8% among HBsAg negative and anti-HBc positive individuals, and 9.2% among HBsAg negative healthy blood donors. Regarding to the high rate of samples containing HBV-DNA from blood donors may be attributed to sensitivity of RT-PCR test (10 copies/ml) or may be to mutation in pre-s/s gene. Similar to this result was reported in collaborative study was undertaken to explore the molecular basis of OBIIs prevalent in Europe, Ninety-one percent (91%) of 77 donor samples of European origin HBV DNA positive and HBV surface antigen (HBsAg) negative were confirmed. Viral load ranged between unquantifiable and 5640 IU/mL (median 25 IU/mL) [59].

Another study conducted in Germany among 5300 individuals randomly selected from an 18- to 70-year-old population established 8.7% HBV prevalence: 0.6% HBsAg-positive, 6.7% anti-HBs-positive, and 1.4% isolated anti-HBc-positive. They investigated 153 anti-HBc-positive individuals for HBV DNA and found 60 of them (39.2%) to be positive. This ratio was higher in patients infected with HIV and HCV. Isolated anti-HBc positivity and HBV DNA positivity may be more elevated in areas with high hepatitis B endemicity [60], despite of another study conducted in Hong Kong showed a contrast result, where was indicated that the prevalence of OHB (0.13% and 10 out of 11 OHB samples were positive for anti-HBc, with extremely low level of DNA "14.1 IU/ml") was low even in area of high endemicity for HBV (8%) (53). A recent study demonstrate that the prevalence of occult HBV infection was high (12.5%)
in the Solomon Islands, a highly endemic region for HBV infection, and 96% of the carriers with occult HBV infection were anti-HBc positive[61].

4.2 Conclusion

The prevalence of occult HBV among anti-HBc positive Palestinian blood donors was high; rejection of anti-HBc positive blood units will be beneficial to decrease the risk of HBV transmission. Therefore, introducing anti-HBc antibodies testing in the routine screening in blood bank centers will minimize the possibility of HBV transmission with its potential consequences particularly in immunocompromised recipients, taking into consideration the cost effectiveness of this test in comparison to the real time PCR.

4.3 Recommendations

- A high prevalence of anti-HBc antibodies was found among blood donors highlighted the need to develop new strategies for blood donors screening for hepatitis B virus through:
  
  i. Integrating all blood banks in the West Bank into one national center in Ramallah with one branch in the north, and one in the south to standardize blood testing.

  ii. Increase the proportion of voluntary donors since this will reduce drastically the blood rejection rate and also improve the cost effectiveness of blood collection in Palestine, since a volunteer donor has been found to be the safest source of blood worldwide.
iii. Routine anti-HBc screening of blood donations could probably prevent some transfusion-transmitted HBV infections. Therefore, anti-HBc antibody should be tested routinely on blood donors.

iv. The implementation of nucleic acid testing (NAT) for donor screening to reduce the residual risk of transmitting HBV through blood transfusion by allowing the detection of infections in the so-called pre-seroconversion window period (before the onset of HBsAg) as well as the detection of OBIs, both characterized by the presence of HBV-DNA in the absence of HBsAg.

v. It is strongly recommended to utilize a highly sensitive and specific approach based on “nested” or “real time” PCR techniques and the use of oligonucleotide primers specific for different HBV genomic regions and complementary to highly conserved (genotype shared) nucleotide sequences.
References


(60). Duygu F, Ugur A, Mahmut B. Determination of hepatitis B virus DNA incidence, viral load, and mutations in blood donors with

Appendices
Appendix "A"

Questionnaire on the prevalence of hidden hepatitis B in blood donors

This study was designed to determine the health status of blood donors, particularly with regard to hepatitis B, which leads to health problems reflect on the donor and the beneficiary of the unit of blood, all results will remain confidential and keeps you the option to participate in this study, note that your participation will contribute to the development of work in blood banks.

Signature:-----------

• Personal information:

1. Serial Number: _______ sample number: _____________ (for laboratory)

2. Date: ___ / ___ / __________

3. Name: _____________________________

4. Gender: ( ) Male    ( ) Female

5. Age: _____________

6. Place of birth: Palestine outside of Palestine, select: ____________

7. The province and place of residence: ___________

8. Place of residence :( ) City   ( ) village   ( ) camp
9. Phone Number (Cell): ______________

10. Occupation: ( ) student  ( ) work  ( ) does not work

11. Select the type of work: ______________

12. Qualifications: ( ) illiterate  ( ) primary
    ( ) secondary  ( ) university

13. Marital status:  ( ) Single  ( ) Married

14. Monthly Income:  ( ) > 1000
    ( ) 1000 -3000  ( ) <3000

• Medical information:

1. Reason for blood donation: family replacement  ( ) Yes  voluntary  ( ) No

2. Donation No.: For the first time ( )  for several times ( )

3. Have you taken a taste of hepatitis?  ( ) Yes  ( ) No

4. How many doses?  ( ) 1  ( ) 2  ( ) 3

5. Have I got an infection and liver or yolk?
    ( ) Yes  ( ) No  ( ) I do not know
6. If yes what kind? ______________

7. Do you suffer from chronic diseases?  
   ( ) Yes  ( ) No

8. If the answer is yes, what is the disease? ______________

9. Are you taking any current treatment for a chronic illness?
   ( ) Yes  ( ) No

10. If the answer is yes, what is the treatment? ________________

11. Did you drink alcohol before?  
    ( ) Yes  ( ) No

12. Is the partner carrier of the hepatitis B virus?  
    ( ) Yes  ( ) No

13. Is there among the family members infected with hepatitis?
    ( ) Yes  ( ) No  ( ) I do not know

• Risk factors:

1. Have you participated one shaving tools?  
   ( ) Yes  ( ) No

2. Have you ever just taken a tattoo?  
   ( ) Yes  ( ) No

3. Do you live with a patient has a lack of immunity?  
   ( ) Yes  ( ) No

4. Do you live with a patient who receives blood units periodically?
   ( ) Yes  ( ) No

5. Is the transfer of units of blood to you before?  
   ( ) Yes  ( ) No
6. Do you have a history of surgery before? ( ) Yes ( ) No

7. Have you ever dealt with intramuscular injection or intravenous?
   ( ) Yes ( ) No

8. Do you have a history of teeth pulling or cleaning teeth?
   ( ) Yes ( ) No

9. Have you ever been exposure to needle contaminated with the blood of someone? ( ) Yes ( ) No

10. Is there a family college or dialysis patient has a kidney transplant?
    ( ) Yes ( ) No

   Thank you for your cooperation
Appendix "B"

استبيان عن مدى انتشار التهاب الكبد البائي الخفي لدى المتبرعين بالدم

صممت هذه الدراسة لمعرفة الوضع الصحي للمتبرعين بالدم، خاصة فيما يتعلق بالتهاب الكبد البائي، والذي يؤدي لمشاكل صحية تتعكس على المتبرع والمستفيد من وحدة الدم، كافية النتائج ستبقى سرية ويبقى لكم خيار المشاركة في هذه الدراسة، علماً بأن مشاركتكم ستساهم في تطوير العمل في بنوك الدم.

المعلومات الشخصية:

1. الرقم المتسلسل:_________ رقم العينة:______________ (خاص بالمختبر)

2. التاريخ:__/__/________________

3. الاسم:____________________

4. الجنس: ( ) ذكر ( ) أنثى

5. العمر:_____________

6. مكان الولادة: ( ) فلسطين ( ) خارج فلسطين حدد:______________

7. المحافظة ومكان السكن:_____________

8. مكان الإقامة: ( ) مدينة ( ) قرية ( ) مخيم

9. رقم الهاتف (الخيلوي):_____________

10. العمل: ( ) يعمل ( ) لا يعمل ( ) طالب

11. عدد نوع العمل:_____________
12. المؤهل العلمي: ( ) أمي ( ) أساسي ( ) ثانوي ( ) جامعي

13. الحالة العائلية: ( ) أعزب ( ) متزوج

14. الدخل الشهري: ( ) >1000 ( ) 1000-2000 ( ) 2000-3000 ( ) <3000

معلومات طبية:

1. التبرع بالدم: تطوع لصلة عائلية غير ذلك حدد:

2. متبرع بالدم: ( ) لأول مرة ( ) لعدة مرات ( ) لا

3. هل سبق وأخذت طعم التهاب الكبد؟ ( ) نعم ( ) لا

4. ما عدد الجرعات؟ ( ) 1 ( ) 2 ( ) 3 ( ) لا أعرف

5. هل سبق وأصيبت بالتهاب كبد أو صفارة؟ ( ) نعم ( ) لا ( ) لا أعرف

6. في حال الإجابة بنعم ما نوعة?

7. هل تعاني من أمراض مزمنة؟ ( ) نعم ( ) لا

8. في حال الإجابة نعم ما هو المرض?

9. هل تتناول أي علاج حاليا لمرض مزمن؟ ( ) نعم ( ) لا

10. في حال الإجابة نعم ما هو العلاج?

11. هل تناولت الكحول سابقا؟ ( ) نعم ( ) لا
12. هل الزوج/ة حامل لفيروس التهاب الكبد؟ ( ) نعم ( ) لا
13. هل يوجد بين أفراد العائلة مصاب بالتهاب الكبد؟ ( ) نعم ( ) لا أعرف

عوامل خطورة:

1. هل سبق وشاركت احدا بأدوات الحلاقة؟ ( ) نعم ( ) لا
2. هل سبق وأن تعاطيت الوشم؟ ( ) نعم ( ) لا
3. هل تعيش مع مريض لديه نقص بالمناعة؟ ( ) نعم ( ) لا
4. هل تعيش مع مريض ينقل له وحدات دم دوريamente؟ ( ) نعم ( ) لا
5. هل تم نقل وحدات دم لكم سابقا؟ ( ) نعم ( ) لا
6. هل يوجد لديكم قصة عمل جراحي سابقة؟ ( ) نعم ( ) لا
7. هل سبق وتناولت حقن عضليا او وريدية؟ ( ) نعم ( ) لا
8. هل لديكم قصة خلع اسنان او تقويم او تنظيف اسنان؟ ( ) نعم ( ) لا
9. هل سبق وتعرضت لوصفة ابرة ملونة بدم شخص ما؟ ( ) نعم ( ) لا
10. هل يوجد بالعائلة مريض غسيل كلية او لديه زراعة كلية؟ ( ) نعم ( ) لا

شكرا لتعاونكم
لـفيروس التهاب الكبد الوبائي نوع DNA (ب) عند المتبرعين بالدم في نابلس - شمال الضفة الغربية

إعداد
أميرة ماجد شيخة

إشراف
د. أدهم أبو طه
د. كمال الضميدي

قدمت هذه الأطروحة استكمالًا لوثائق الحصول على درجة الماجستير في الصحة العامة من كلية الدراسات العليا في جامعة النجاح الوطنية في نابلس-فلسطين.

2011م
للفيروس التهاب الكبد الوبائي نوع (ب) عند المتبرعين بالدم في نابلس - شمال الضفة الغربية

الطريقة البحث: تم اختيار 500 عينة من المتبرعين بالدم اثنتي التحاليل المسحيّة خلوى من HBsAg، HCV، HIV، وباستخدام تقنية (Elisa) تم فحص الضد الموافق للمستضد الليفي لحمّة التهاب الكبد الوبائي، ثم تم

المتبرعون

 sistematically

الرعى

أ. أبو طه

د. كمال الضمدي

الملخص

مدخل: الكشف عن الحمض النووي لحمّة التهاب الكبد الوبائي (HBV-DNA) من دون وجود المستضد السطحي (HBsAg) مع أو بدون وجود أضداد فيروس وخارج إطار الفترة الشباكيّة للطور الحاد يعرف بالتثبيت الوبائي الخفي (OHB).

وقد تم توثيق انتقال عدوى بالتهاب الكبد الوبائي من متبرعين لدم أو أعضاء سلبيي المستضد السطحي HBsAg، بينما كان الضد الموافق للمستضد الليفي لحمّة التهاب الكبد الوبائي (anti-HBe) إيجابيا لديهم. ولذلك فإن الوقاية من المخاطر المتبقية من انتقال حمّة التهاب الكبد الوبائي تعتزم في معظمها على الفحص المسحي الكيميائي للضد الموافق للمستضد الليفي لحمّة التهاب الكبد الوبائي مع/أو فحص الدنا لدى المتبرعين بالدم .

الهدف من هذه الدراسة: إن الهدف من هذه الدراسة هو تحديد مدى انتشار أضداد المستضد الليفي لحمّة التهاب الكبد الوبائي لدى المتبرعين بالدم في فلسطين, وذلك لتقييم وتيرة الدنا anti-HBe (حمّة التهاب الوبائي) عند المتبرعين بالدم حملة ضد المستضد الليفي (HBV-DNA) ضمن مراكز نقل الدم في مدينة نابلس شمال الضفة الغربية.

طريقة التحليل: تم اختبار 500 عينة من المتبرعين بالدم اثنتي التحاليل المسحيّة خلوى من HBsAg، HCV، HIV، 1،2 وفحص الضد الموافق للمستضد الليفي لحمّة التهاب الكبد الوبائي، ثم تم
التحري عن الدنا عن طريق تفاعل البلمرة المتسلسل (RT-PCR) وذلك للعينات الإيجابية لفحص (anti-HBc).

النتائج: وفقاً للفحص المسحي الروتيني، تم رفض عينة 11 عينة بنسبة 2.2% (4/0.8) عينات إيجابية لـ HBsAg، و 1 عينة إيجابية لـ HCV، و 6 عينات لديها مشاكل صحية. من بين وحدات الدم المقبولة للمتبرعين كان معدل انتشار الدخان الموافق للمستضد anti-HBc الليفي 49/489 (10.1%) بينما كان 92% من المتبرعين بالدم كشف لديهم RT-PCR عن طريق تفاعل HBV-DNA (13.5 ×10 مكعب / نسخة / مل).

الاستنتاجات: إن العدوى المحتملة لهذه الوحدات مختلفة عليه، ولكن استخدامها لا يمكن اعتباره آمناً على الأقل في المرضى الذين يعانون ضعف المناعة. يشار إلى تطوير الفحص المسحي الروتيني للمستضد السطحي HBsAg للتغلب على أوجه القصور في حساسية الكشف عن HBsAg في الأشكال المتحولة من فيروس الكبد، بالإضافة إلى تطبيق فحص نوعي ضد المستضد الليفي anti-HBc للفحص روتيني يملأ على القضاء على الوحدات المخموحة بعدوى التهاب الكبد البائي، مع الأخذ بعين الاعتبار مدى فعالية التكلفة لهذا الفحص بالمقارنة مع فحص RT-PCR.