An-Najah National University
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HBV Status among Vaccinated Family Members of HBV Positive Carriers in Northern Palestine (Tulkarm District)

By

Khalid Amin Abu-Khater

Under the supervision of

Dr. Nael Abu-Hasan and Dr. Kamel Adwan

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and approved by

Committee Mebmers: Signature

1. Dr. Nael S. Abu-Hasan (Advisor)
2. Dr. Kamel Adwan (Advisor)
3. Dr. Ghassan Abu-Hijleh (External examiner)
4. Dr. Awni A. Abu-Hijleh (Internal examiner)

DEDICATION

To my beloved wife,

Mother's soul (mercy be upon here),

Father and Sons with all love

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Abstract

The present study was designed to evaluate the status of Hepatitis B Virus (HBV) and the efficacy of the vaccination programme among a total of 161 high-risk family members of hepatitis B virus carriers who were subjected to the governmental vaccination programme in Tulkarm, Northern Palestine, 1997-1998. The seroprevalence of hepatitis B surface antigen (HBsAg)-positivity was (8.1%). The highest seroprevalence rate found among the 21-30 age group(7.5%), followed by the 11-20 age group and the <10 age. Differences in the prevalence rates among the various age groups were not statistically significant ($P > 0.05$).

Significant differences were observed in the prevalence of rates of HbsAg positive in males (92%, $P = 0.008$). Although differences in the prevalence rates of HBV infection according to residence were not statistically significant ($P = 0.07$) residence in the village was clearly associated with higher rate of HBV infection (84.6%) compared to that in town and camp.

Nine (69.2%) of the infection transmission cases attributed to brother-brother relationship and 4 (30.8%) to father-children relationship. In small families (4 or less), the mean rate of HBV

infection was 53.8%, whereas in large families (more than 4) it was 46.2%.

Anti-HBV titers below the protective level (1000 IU/L) were found in all HBV-infected family members. Data analysis regarding family members with anti-HBV above the protective level, revealed no significant difference between groups completed the vaccination dose schedules (3-doses) and those received one or two doses ($P > 0.05$). However median titers of anti-HBV were higher in the group receiving 3-doses than in corresponding group receiving 1 or 2 doses.

During follow up after 6 months from testing date, three additional HBV infection was observed. On the other hand, HBsAg clearance was observed in four cases among those found to be HBsAg positive in the initial stage of the study.

1.1 General Introduction

The disease known as hepatitis B is caused by the infectious Hepatitis B virus (HBV). HBV alone is estimated to have infected 400 million people throughout the globe, making HBV one of the most common human pathogens. Hepato-cellular carcinomas (HCC), one of the most common cancers afflecting humans, is primarily caused by chronic HBV infection. In the last few decades, the correlation between HBV and the development of HCC has been well established. However, the mechanism by which HBV transforms hepatocytes remains elusive. Before HBV can transform a cell, the virus must first infect it. However, the mechanism through which HBV invades hepatocytes has not been resolved despite further understanding of the viral proteins involved. Vaccines are available against HBV, but they may not be 100% effective against all variants of HBV. Furthermore, there is no cure for individuals already infected. Much more research is needed before we fully understand and control the spread of this infectious agent.

Viral hepatitis is the term reserved for infections of the liver by one or more of the distinct hepatitis viruses. The terms, hepatitis A and

hepatitis B, were first introduced by MacCallum in 1947 in order to categorize infectious (epidemic) and serum hepatitis.[1]

Before the viruses causing hepatitis were isolated, transmission was differentiated on the basis of epidemiological observations. Type A hepatitis was considered predominantly transmitted via the fecal-oral route while type B hepatitis believed to be primarily transmitted parenterally. In 1963, Blumberg discovered a previously unknown protein in the blood of an Australian aborigine.[2] This protein was denoted as the Australia (Au) antigen. It became apparent that this protein was related to type B hepatitis. By 1968, other investigators established that the Au antigen (now known as the hepatitis B surface antigen) was only found in the serum of type B hepatitis infected patients.[3, 4]

In 1973 virus-like particles in the serum of patients suffering from type B hepatitis were found.[5] These particles were designated as the hepatitis B virus (HBV). Non-related hepatitis viruses were discovered later, but the hepatitis B virus retained its name.

The viral nature of these particles confirmed by the detecting of an endogenous DNA-dependent DNA polymerase within its core.[6] Discovery of this polymerase allowed the detection and characterization of the HBV genome.[7] The HBV genome is unique in the world of viruses due to its compact nature, overlapping reading frames, and

dependence on a reverse-transcriptional step, though the virion contains primarily DNA. In light of this, the human hepatitis B virus became the archetype of the hepadnavirus family, *Hepadnaviridae*.

Though the hepatitis B surface antigen (HBsAg) from HBV has been detected in other primates, humans remain its primary reservoir. HBV has been estimated by the World Health Organization (WHO) to infect over two billion people worldwide. Approximately 500 million are chronic carriers. Transmission of HBV is primarily through blood and/or sexual contact, though other methods of transmission have been suggested. The large reservoir of infected individuals has sustained a satellite virus known as the hepatitis D virus (HDV). HDV can only replicate in cells already infected with HBV since HDV uses hepatitis B surface proteins to package its own RNA.

1. 2 Epidemiology

HBV is worldwide in distribution and there are considerable differences in the prevalence of infection in different geographical areas even within the same country. The prevalence of HBV has been decreasing in developed countries due to the availability of the hepatitis B vaccine, increasing knowledge of how the virus spreads as well as screening of donated blood before use.

The hepatitis B virus is primarily found in the blood of infected individuals. Virus titers, as high as ten billion virions per milliliter of blood, have been reported in HBe-positive carriers. However, HBV has also been detected in other bodily fluids including urine, saliva, naso-pharyngeal fluids, semen, and menstrual fluids.[8,9]

Transmission of HBV occurs most efficiently via percutaneous introduction (i.e. needle stick injury). Sexual transmission is also possible though inefficient. There are other potential routes of transmission, but their efficiency is not easily measured. Children of mothers with active HBV are also at risk of acquiring HBV. Uninfected individuals living with an HBV carrier are at greater risk of contracting HBV than those not living with a carrier. This is likely due to the fact that HBV can survive even on a dry surface for over a week.[10] However, it should be remembered that for HBV to infect, it still must gain entry into the bloodstream of an uninfected individual.

Higher Risk Groups include: individuals living in close proximity to a known infected individual, users of intravenous drugs, particularly those who share their needles, individuals who have multiple sexual partners, health care workers, daycare workers and anyone who may come into contact with human body fluid from an unknown or known HBV carrier source.

1. 3 HBV Vaccines

Three HB vaccines have been commercially marketed and available. The original Heptavax B®, a plasma-derived product, was introduced in the international market in 1982. This vaccine is available only in limited amounts and reserved for patients with specific medical conditions. The other two preparations are recombinant vaccines prepared from yeast cultures (*Saccharomyces cerevisiae*) that have been genetically altered to produce the hepatitis B surface antigen. Recombivax HB® was the first vaccine created using recombinant DNA technology and has been available since 1987. In 1989 a Belgium firm produced Engerix-B® vaccine.

1. 3. 1 Vaccination dose Schedule

For long-term protection a regimen of three doses (given at 0, 1, and 6 months) is recommended and it is usually given intramuscularly in the arm (deltoid muscle). About 96% of young, healthy adults "seroconvert" after completing the vaccine series and form antibodies to the hepatitis B surface antigen (anti-HBs). More specifically, when administered in a three-dose regimen, Recombivax HB has been shown to induce protective antibody synthesis in more than 99% of healthy adults 20-39 years of age. Healthy adults who received either

Recombivax HB (10µgm/dose) or the plasma-derived Heptavax B (20µgm/dose) demonstrated equal seroconversion rates.

1. 3. 2 Post-test-vaccination

Post-test-vaccination is recommended for healthcare providers and should be performed one to six months after the final injection. Some people do not respond to the initial vaccination series, and the only way to know this is to test for the anti-HBs. If antibodies have not developed, a fourth vaccine dose should be given with re-testing after one month. If antibodies still not be present, the three-dose series should be repeated with the dose given the previous month considered as the initial dose of the series. Approximately half of the non-responders to the first series will respond to a second three-injection regimen. If testing is delayed beyond six months, and particularly if it occurs years after completion of the series, interpretation of the results is much more difficult. A person testing negative at this time may be a non-responder or simply a responder who has lost detectable antibodies but is still protected from contracting the clinical disease as a result of their immunological memory. In summary, vaccination post-testing should be scheduled one to six months after the last inoculation.

1. 3. 3 Booster injections

Protection lasts at least ten years due to the anamnestic response. This is based on the observation that no one receiving the original vaccine who was immunocompetent and responded has developed clinical hepatitis B. Individuals who have lost serologically detectable anti-HBs have developed a secondary anamnestic humoral response that protects against clinical infection when challenged by live HBV via natural exposure or by an additional dose of vaccine. Ongoing studies continue to assess the duration of immunological memory and recommendations will be updated as appropriate.

1. 3. 4 Plasma-derived vaccine/recombinant vaccine

Both the plasma-derived Heptavax B and the two recombinant vaccines use hepatitis B surface antigen. Thus, persons who originally received the plasma-derived product can use either recombinant vaccine as a booster.

1. 3. 5 Family immunization

Currently the Central Disease Control (CDC) recommends that all infants be immunized at birth for hepatitis B. Previously unimmunized teenagers should also be immunized before they begin behaviors that

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have also been found in extra hepatic sites. These include mononuclear cells, bile duct epithelial, endothelial, pancreatic acinar cells, and smooth muscle tissue. They have also been found in adrenal glands, gonads, cultured bone marrow, kidneys, lymph nodes, spleen, and thyroid glands of acute hepatitis B infected patients.[11]

1.5 Diagnosis

Acute viral hepatitis infection can be broken into four stages:

a) Incubation period, which is the time between initial viral entry into the cell to first day of symptoms;

b)Prodromal or pre-icteric period;

c)The icteric phase

d) Recovery

Symptoms during the onset of acute hepatitis B viral infection vary, depending on the individual. Many children and some adults infected with the virus never show any discernible symptoms. However, most infected individuals experience a certain level of jaundice, which tends to develop soon after the virus can be detected in the blood. Often, jaundice is preceded by mild fevers, fatigue, and malaise, loss of appetite, and sometimes nausea and vomiting.

During the icteric or blood-borne phase, an infected individual's urine tends to have a dark, golden-brown appearance. The lightening of the

stool as well as the yellowing of the skin and the sclera as seen typically in jaundice often follow this. Jaundice is considered clinically apparent once total bilirubin levels are greater than 2-4mg/dl.

There are some common signs that suggest one may have a liver problem. However, not all-liver problems are attributable to the Hepatitis B virus. Hepatitis A, C, D, E, G viruses, as well as alcohol, chemical, bacterial, and other conditions can damage one's liver. The followings are some of the common symptoms of liver problem; yellow discoloration of skin and/or eyes, abdominal swelling or severe abdominal pain, prolonged itching of the skin, very dark urine, pale stools, passage of bloody or tar-like stools, chronic fatigue, nausea and loss of appetite.

1. 5. 1 Serological tests for diagnosing hepatitis B

Many serological tests have been developed with considerable variations in their sensitivity to differentiate the type of viral infection as well as discriminate between chronic and acute hepatitis B virus (HBV) infection. The most sensitive and specific methods used commercially in diagnosis are radioimmunoassays (RIA) and enzyme-linked immuno-sorbent assays (ELISA). Both assays make use of specific antibodies against various HBV proteins and can detect HBsAg

proteins as low as 0.25 ng/mL and anti-HBs antibodies at a level of 1 mIU/mL. Figure 1 shows the various seriological events during the acute phase of HBV infection.

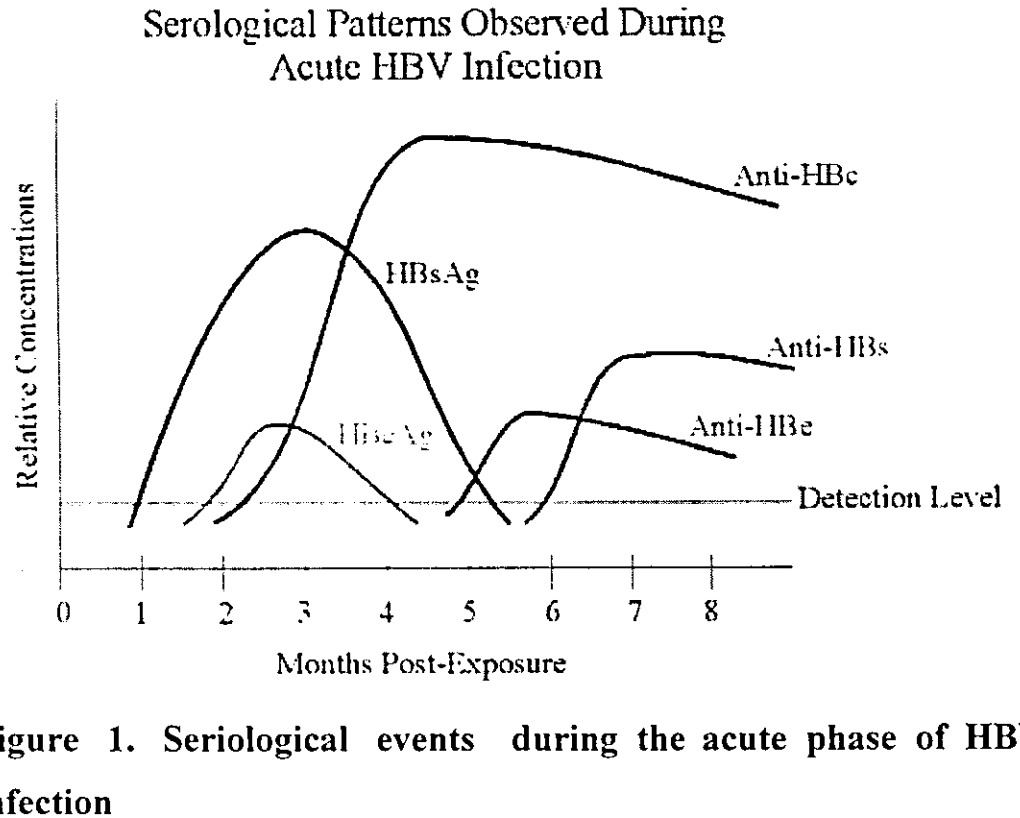

Figure 1. Seriological events during the acute phase of HBV infection

Polymerase chain reaction (PCR) has also been used in detecting low levels of HBV DNA present in both blood and liver tissue samples. Data presented in Table 1 shows the interpretation of the assay results based on the presence of the various expressed antigens.

Table 1. Interpretation of assay results for HBV status

Presence of HbsAg	Presence of Anti-HBs	Presence of Anti-HBc	Interpretation of Assay Results
Positive	Negative	Negative	These results are characteristic of early acute HBV infection.
Positive	Positive or Negative	Positive	These results suggest either acute or chronic HBV infection, which may be differentiated with respect to IgM anti-HBc.
Negative	Positive	Positive	These results are characteristic of previous HBV infection and current immunity to the virus.
Negative	Negative	Positive	These results do not have a clear interpretation. They could be due to HBV infection in the remote past, low-level HBV infection, or false-positive/ non-specific reactions. If present, anti-HBs help validate anti-HBc reactivity.
Negative	Negative	Negative	These results suggest that liver toxicity is due to some other agent other than HBV.
Negative	Positive	Negative	These results are typical of a vaccinated individual.

1. 5. 2 Biochemical tests

AST (Aspartate Aminotransferase) or SGOT (Serum Glutamic-Oxaloacetic Transaminase): 5-54 U/L, tissues damage releases this enzyme and elevated levels can be detected in the blood.

ALT (Alanine Aminotransferase) or SGPT (Serum Glutamic-Pyruvic Transaminase): 0-36 U/L, this enzyme is found mainly in the liver, but can also be seen in lower amounts in heart, muscle, and other tissues. Increased levels of this enzyme in the blood can be attributed to liver damage, kidney infection, or cardiac infarction.

Other enzymes and compounds tested for HBV infection include alkaline phosphatase, GGT (Gamma Glutamyltransferase): LDH (Lactose Dehydrogenase), albumine and bilirubin.[12]

1. 6 Clinical sequale of HBV infection

1. 6. 1 Acute hepatitis

The clinical course of HBV runs similarly to that of Hepatitis A Virus (HAV), but tends to be more severe and may be associated with serum sickness like syndrome. The mildest attacks are asymptomatic and are detectable only by an increase in serum transaminase levels. Alternatively, patients may be anicteric, but may suffer from gastrointestinal and influenza-like symptoms. These patients are likely to remain undiagnosed unless a clear history of exposure is available.

The severity of infection may vary from the asymptomatic and icteric (from which recovery is typical) through to fulminant, fatal viral hepatitis.

Icteric attacks in adults are marked by a prodromal period (typically three to four days, but may last up to two or three weeks) during which patients feel sickly, suffer from digestive symptoms such as anorexia and nausea and may, in the later stages, have mild pyrexia. Other common symptoms are rigors, loss of desire to drink alcohol or smoke, malaise, and occasionally, severe headaches. The prodromal period is followed by the darkening of urine and lightening of feces, followed by the development of jaundice. [13]

1.6.2 Cirrhosis

Cirrhosis can be characterized anatomically by widespread nodules in the liver combined with fibrosis. These excessive nodules and fibroids result in the distortion of the normal liver architecture and interfere with blood flow through the liver. Cirrhosis may also result in the inability of the liver to perform its functions as abnormalities progressively develop.

1. 6. 3 Fulminant hepatitis

This is a rare form of the disease, which usually overwhelms the patient within ten days. This form may develop so quickly that jaundice is inconspicuous and may be confused with acute psychosis or meningo-encephalitis. On the other hand, the patient may become deeply jaundiced. Foreboding signs may be repeated vomiting, fetor hepaticus, confusion, and drowsiness. These are then supervened by coma, indicating likely acute liver failure. The patient temperature rises, jaundice deepens, and liver shrinks, possibly accompanied by widespread hemorrhages.

The levels of serum bilirubin and ALT are poor prognostic indicators because ALT levels may actually decrease as the patient's clinical condition worsens. Prothrombin is the best indicator of prognosis. Frequency of the fulminant course varies, depending upon the type of viral hepatitis and prevalence of hepatitis B carriage.[14]

1. 6. 4 Hepatocellular carcinoma (HCC)

Hepatocellular carcinoma is the technical term for liver cancer. This form of the disease develops after a long time in individuals suffering from chronic hepatitis B infection. The events that trigger the development of this disease are currently unknown.

1. 7 Treatment

1. 7. 1 Acute infection

In general, there are no specific treatments for benign acute viral hepatitis. The use of adreno-corticosteroids, recommended by some, appears to have no effect curing the underlying disease. Furthermore, it appears that use of steroids in early treatment of hepatitis B virus (HBV) infection may result in the development of a persistent infection. Therapeutic effectiveness of interferon use on the prognosis and course of acute HBV infection remain unknown.[15]

1. 7. 2 Chronic infection

A number of elements have been used in the treatment of chronic HBV in order to eliminate its infectivity, transmission, arrest the progression of liver disease, improve the clinical prognosis, and to prevent the development of hepatocellular carcinoma (HCC). Currently, there are several treatments being used. Interferon alpha use is most common, but now lamivudine (3TC) and others are being looked at as potential therapeutic agents.[16, 17, 18] Combinations of antiviral drugs are being used with some success. These include: Acyclovir, Adefovir Dipivoxil, Adenine Arabinoside, Famciclovir, Ganciclovir Lamivudine and

Lobucavir. None of these treatments can be called a cure. A true cure for this disease still remains elusive.

1. 8 Prevention

All blood and body fluid should be treated as if they contain HIV, HBV and other blood-borne pathogens. Gloves should be used when handling suspicious materials. Hands should be washed regularly. Also, needles and other sharp objects should be handled with extra care to prevent unwanted needle-stick transmission. Suspected contaminated surfaces should be disinfected and cleaned. Surfaces can be decontaminated using disinfectants such as 0.5% sodium hypochlorite, 2% aqueous alkalinized glutaraldehyde, quaternary ammonium germicides or phenolic-based disinfectants.

1. 9 Vaccination

Currently, the best method to prevent HBV infection is through vaccination. The most common vaccine on the market is derived from a recombinant yeast source. The small hepatitis B surface protein (SHBs) is generated by yeast cells. Expression of this protein by yeast results in SHBs particle formation. However, particles are not secreted by the yeast. Disruption of yeast cells is performed in order to liberate

the produced spheres into solution. These particles are then purified through clarification, ultra-filtration, chromatography, and ultra centrifugation. The purified particles are then adsorbed onto aluminum hydroxide to which thimerosal is added to preserve the solution.

On July 8, 1999, the American Academy of Pediatrics (AAP) and the U.S. Public Health Service (PHS) jointly recommended reducing infant exposure to thimerosal, a commonly used vaccine preservative that contains mercury.[19, 20] Specific recommendations were made to postponed the first hepatitis B vaccine dose until 2-6 months of age for infants born to hepatitis B surface antigen (HBsAg)-negative (i.e., not hepatitis B virus [HBV]-infected) women.[19, 20] Infants born to HBsAg-positive (i.e., HBV-infected) women, or to women whose HBsAg status was unknown, were recommended to receive postexposure prophylaxis with the first dose of hepatitis B vaccine administered within 12 hours of birth.[19, 20] By mid-September 1999, when adequate supplies of preservative-free hepatitis B vaccine became available, PHS advocated a return to previous infant hepatitis B vaccination practices, including administering the first dose of hepatitis B vaccine to newborns in hospitals that had discontinued the practice.[21]

The two yeast-derived vaccines licensed in most countries are Engerix-B (SmithKline Beecham, Philadelphia, PA) and Recombivax HB

(Merck & Co., West Point, PA). Both products are structurally and chemically similar with less than 2% yeast protein remaining in solution. Recombivax HB, however, is treated with formaldehyde before its adsorption onto alum. As both are yeast-derived, the S-protein is not glycosylated (as yeast does not possess the correct post-translational machinery to do so). Both appear to be quite effective as vaccines, allowing for immunization against the various forms of HBV. The vaccines, however, should not be frozen, as this appears to be deleterious to its immunogenicity. Studies have shown that freezing of these vaccines results in lower immune response.

There are also some other forms of immunization and vaccines, but the ones mentioned above generally appear to be the most effective and the most widely used.

The typical vaccination regimen is a 3 doses of HBsAg, injected intramuscularly at intervals of 0,1 and 6 months or 0, 1, 2 and 10 months. The 0, 1 and 6 month vaccination regimen is preferred for routine pre-exposure prophylaxis. The four-dose regimen may be preferred, however, for immuno-compromised patients or for postexposure prophylaxis. It has also been recommended that a booster shot be given every 5 to 7 years after the initial vaccination. Infants may also be vaccinated in this way.

However, there have been some (though rare) reports of adverse reactions to these yeast-derived vaccines. Some possible serious adverse reactions include skin, rheumatic, vasculitic, ophthalmologic, hematologic, and neurologic reactions. In general, the side effects of HBV vaccination are relatively minor such as pain at the injection site (3% to 29%) and temperature greater than 37.7C.

Also, not everyone responds adequately to the first set of vaccination injections. Especially for those working in the health profession or those who are in close contact with someone who is infected, it is advisable to be tested about a month or so after the final vaccination to ensure antibody titers are high enough.

There has been a recent surge in development of new hepatitis B vaccines made of the large and small hepatitis B surface proteins or DNA vaccines or even combination vaccines such as a vaccine providing dual protection against hepatitis A and B (Twinrix, SmithKline Beecham Biologicals).

1. 10 Hepatitis B genome

Electron microscopy gave the initial views of the hepatitis B genome. In virions, the genome appears to be circular, partly double-stranded of

approximately 3200 nucelotides in length .[22] There are at least seven major subtypes of HBV, distinguished by sequence differences in the surface antigen gene.[23] Unlike others, HBV virions contain both DNA and RNA. Moreover, some regions of the packaged genome can be single stranded, double stranded or even triple stranded.

HBV genome consists of four defined overlapping open reading frames (ORFs) which result in the transcription and expression of the seven different hepatitis B proteins. The four ORFs lead to the transcription and translation of seven different HBV proteins through use of varying in-frame start codons. ORF P occupies the majority of the genome and encodes for the hepatitis B polymerase protein. ORFs encodes the three surface proteins.[24] ORF C encodes both the hepatitis *e* and core protein.[25] ORF X encodes the hepatitis B X protein.[26, 27]

Multiplication of the HBV genome occurs within the nucleus of an infected cell. RNA polymerase II transcribes the circular HBV DNA into greater-than-full length mRNA. Once produced, the genomic RNA exits the nucleus and enters the cytoplasm whereupon it can be translated to generate the HBV polymerase, core and e proteins.

At present 6 major genotypes (A,B,C,D,E,F) are recognized. Their worldwide distribution as follows: **Group A** - Orig - N. Europe - Sub-Saharan Africa; **Group B** - Confined to - Eastern Asia (China); **Group**

C - Far East (Japan); **Group D** - Mediterranean - Near, Mid East, South Asia; **Group E** - W. Sub-Saharan Africa, south to Angola and **Group F** - New World - Brazil, N. and S. America

Four subtypes of the hepatitis B surface antigen (HBsAg) have been observed. These are defined by two mutually exclusive determinant pairs d/y and w/r with a common determinant 'a'. These subtypes are adw, ayw, adr, and ayr.

Four genomic groups of HBV were later referred as genotypes designated with A-D. Sequencing of the S-gene of HBV is the molecular basis for the assessment of the serological variations of HBsAg within the major four subtypes. Two new genotypes of HBV are designated with E and F. The F genotype diverges from other HBV genomes sequenced by 14%. So far, it is the most divergent HBV genome. Worldwide molecular epidemiology of HBV is based on the variability of the S-gene. The E and F strains appear to originate from aboriginal populations of Africa in the New World.

1. 11 Life cycle of hepatitis B virus

1. 11. 1 Binding and entry

The hepatitis B virus, as in the case of all other viruses, must first attach specifically onto a cell capable of supporting its replication. Though the liver is the most effective cell type for replicating HBV, other extra-hepatic sites have been found to be able to support replication to a lesser degree. HBV replicative intermediates and/or viral transcripts have been found in mono-nuclear cells[28], pancreatic cells, and smooth muscle tissue, as well as in adrenal glands, gonads, kidneys[29, 30] and many other organs of acute hepatitis B infected patients. Viral attachment often determines host and tissue specificity of a virus. However, for HBV, there are no cell-lines available that are able to support viral replication. Only primary duck hepatocytes, which are freshly explanted from the liver, can support DHBV infection.[31, 32, 33] Consequently, the initial steps of HBV entry is poorly understood. However, several differentiated and immortalized cell lines are capable of supporting viral replication if transfected with viral DNA.[34, 35, 36] The availability of these cell systems has allowed for the elucidation of much of the hepatitis B life cycle. Many proteins have been found associated with the various hepatitis B surface proteins. Despite these

findings, the proteins and mechanism of HBV entry into cells has yet to be well characterized.

The immediate steps following HBV entry are not clearly defined. Some scientists think that the nucleocapsid must be released from the envelope proteins. Other studies suggest that a proteolytic event must occur on the large surface protein to expose a membrane fusion domain.[37] However, it is believed that this occurs at the plasma membrane and not within an acidic vesicle.[38]

After uncoating, the nucleocapsid is believed to be transported to the nuclear membrane. The DHBV system has suggested that HBV genome uncoating occurs at the nuclear membrane.[39]

The HBV DNA is brought into the nucleus where it is repaired to the covalently closed-circular form (cccDNA). Unlike the retroviridae, integration of HBV DNA into host cell DNA is not required for replication. In fact, integration of HBV DNA results in the disruption of one or more HBV ORFs and prevents transcription of functional RNA.

1.12 Aims of the Study

The present study was designed to evaluate the status of HBV and the efficacy of the vaccination programme among a total of 161 high-risk family members of hepatitis B virus carriers who were subjected to the vaccination programme in Tulkarm, Northern Palestine, 1997-1998.

Chapter II

Material and Methods

2.1 Subjects and study design

This study was performed during the 1999-2000 period. Study participants were 161 high-risk family members belonging to 50 families in Tulkarm district. The median age was 23.6 years (range, 3 to 55 years). These family members were designated as high-risk cases, i.e. they were in contact with hepatitis B virus carrier. According to the data collected from reports of the epidemiological service of Public Health Ministry of Palestine, all study subjects received vaccination against HBV during the 1997-1998 period. Only 108 (67.1%) completed the vaccination dose schedules, 50 (31.1%) received 2 doses and 3 (1.8%) one dose. The sera of all study subjects were tested for HBV infection markers, i.e. HBsAg and anti-HBs. The sera were examined by enzyme immunoassay (ELISA). All study subjects were asked to fill out a questionnaire on general data. In the present study, an individual positive for HBsAg was considered "infected" and an individual positive for anti-HBV, and negative for HBsAg was considered "vaccinated". After 6 months of follow up, all the study cases were re-tested for both HBsAg and anti-HBV.

2. 2 Vaccination dose schedules

All subjects were vaccinated against HBV using Hepavax-Gene. Hepavax-Gene is a non-infectious inactivated recombinant sub-unit viral vaccine derived from HBsAg produced in yeast cells using recombinant DNA technology. Doses were administered as described by the manufacturers (Table 2).

Table 2. Hepatitis B vaccination dose schedules

Group	Formulation	Initial dose	1 month dose	6 month dose
Neonates	10mcg/0.5ml	0.5ml (10mcg)	0.5ml (10mcg)	0.5ml (10mcg)
Children up to 10 years of age	10mcg/0.5ml	0.5ml (10mcg)	0.5ml (10mcg)	0.5ml (10mcg)
Adults and older children	20mcg/1.0ml	1.0ml (20mcg)	1.0ml (20mcg)	1.0ml (10mcg)

2.3 Sample collection and processing

2. 3 .1 Sera sample collection

A 5ml venous blood sample was collected from each family member using disposable syringes. The blood was left to clot at room temperature then was centrifuged at 3000rpm for five minutes. Serum was aspirated and two aliquots for each sample were kept in screw-capped vials at – 30°C until testing.

2.3.2 HBsAg detection

HBsAg was detected using Auszyme Monoclonal Diagnostic ELISA kit. The test was performed according to manufacturer instruction as follows:

1-A 200µl, controls or specimens were dispensed into the bottom of the appropriate wells of the reaction tray

2-A 50µl of conjugate solution (anti-HBs bound to peroxidase) was added to the wells and then the tray was gently taped to enhance mixing of conjugate.

3-One bead (coated with mouse monoclonal antibody to HBsAg) was added to each well, and the plate was incubated at 40°C for 75 minutes.

4-The contents of the wells were discharged and the beads were washed three to five times with 4 to 6 ml of distilled or de-ionized water for a total rinse volume of 12 to 30ml.

5-Beads was immediately transferred to properly identified assay tubes.

6-A 300µl of freshly enzyme substrate ODP (O-phenylenediamine.2HCl. Citrate buffer containing 0.02% H_2O_2) were added to each tube including two empty tubes (substrate blanks)

7-Tubes were covered and incubated at room temperature for 30min.

8-A 1000 µl of sulfuric acid stopping solution was added to tubes.

9-Optical densities were then read at 492 nm

2.3.3 Detection of anti-HBsAg

Anti- HBsAg determination was performed using (Bio Elisa kit, S.A anti-HBsAg, Spain) according to manufacture instruction. Testing was performed on diluted sera (1:100) as follows:

1-A 100µl of sera or control samples was added to HBsAg coated wells. Two wells were left empty as blank.

2-The plate was covered with adhesive seal and incubated for 1 hour at 37°C.

3-The contents of the wells were discarded and then washed with diluted phosphate buffer containing 1% Tween 20 and 0.01% thimerosal.

4- A 100 µl of HBsAg conjugated to horseradish peroxidase were added and the plate was incubated for 30 minutes at 37°C.

5- The contents of the wells were discarded and then washed as mentioned in step 3.

6-A 100 µl of tetramethylbenzidine (TMB)- substrate solution were added and the plate was incubated for 30 minutes at room temperature

7- The reaction was then stopped by adding 100µl of stopping solution (1 N sulfate acid)

8- Optical densities were read at 450nm within 30 minutes.

2.4 Statistical analysis

Statistical analysis was performed with the programmer Statistical Package for Social Sciences (SPSS, Chicago). Chi-Square test was used; A $P < 0.05$ was considered statistically significant. Risk factors studied included sex, age, residence and vaccination compliance.

3.1 Study population

In 1994, the Palestinian Ministry of Health started an obligatory HBV vaccination program for high-risk individuals including those with close contact to HBsAg carriers. During the 1999-2000 period, a total of 161 high-risk family members belonging to 50 families in Tulkarm district were included in the present study. According to the data collected from reports of the epidemiological service of Public Health Ministry of Palestine, all study subjects were received vaccination against HBV during the 1997-1998 period. Testing of the family members for the presence of HBV markers revealed that 13 (8.1%) of the 161 family members acquired HBV infection, only 2 (15.4%) of them exhibiting an overt clinical picture of HBV infection.

3.2 Demographic data for the study population

Demographic data for the study population are shown in Table 3. Of these, 71 (44.1%) were females and 90 (55.9%) males. In respect to age, 53 (32.9%) in the < 10, 56 (34.8%) in the 11-20, 40 (24.8%) in the 21-30 and 12 (7.4%) in the > 31 age group. According to the residence, the study population was distributed as follows; town 38(23.6%), village 92 (57.1%) and camps 31

(19.3%). In respect to vaccination dose, 108 (67.1%) received 3 doses, 50 (31.1%) received 2 doses and 3 (1.8%) received a single dose. Dose titers were as recommended by WHO as described previously in the materials and methods section. The anti-HBV titer was below the protective level (1000 IU/L) in 103 (64%) and more than 1000 IU/L in 58 (36%).

3.3 Factors associated with HBV infection

3. 3.1 Age

The distribution of HBV-infected patients according to their age-groups is shown in Table 4. The highest proportion of the infected members 17.5% (7/40) was observed in the 21-30 age group. The age groups 11-20, <10 had 8.9% (5/56) and 1.9% (1/53) cases, respectively. No individual experienced HBsAg +ve in > 31 age group. Differences in the prevalence rates among the various age groups were not statistically significant ($P > 0.05$).

3.3.2 Gender

Data analysis according to sex showed a significant difference in the HBV infection for males ($P = 0.008$). Among the 13 HBV-infected cases, 12 (92%) were males and 1 (8%) was female (Figure 2).

3.3.3 Residence

Only residence in the village was clearly associated with higher rate of HBV infection (Table 5). Out of 13 infected cases only 2 (15.4%) were from the camp, 11 (84.6%) were from the village and none from the town. However, differences in the prevalence rates of HBV infection according to residence were not statistically significant ($P = 0.070$).

3.4 Demographic and serological data for the 13 HBV-infected patients

Data presented in Table 6 present demographic and serological data of the 13 HBV-infected patients and their families. All infected subjects belong to 9 families. None of these subjects was tattooed, hemophiliac, with history of blood transfusion or haemodialysis. Epidemiological survey did not reveal potential risk of HBV infection other than family contact. Nine (69.2%) of the infection transmission cases attributed to brother-brother relationship and 4 (30.8%) to father-children relationship. In small families (up to four members), the mean rate of HBV infection was 53.8% (7/13), whereas in large families (more than four members) it was 46.2% (6/13).

It is worth noting here, that all the 13 HBV-infected family members had anti-HBV titer below the protective level (range = 25–305 IU/L). However, a wide range variation of anti-HBV titer in response to vaccination between different families and even between members in the same family was observed, and exceeded 200 folds in certain families (Table 6). A lower anti-HBV titer was associated with vaccination dose among members of family number 8 (Vaccination dose = 2, median = 110 IU/L, range = 93-136 IU/L). This is in contrast with the results of members of families number 7 and 9 (No. of vaccination dose = 2 each, median = 1068 and 11894 IU/L, range = 127-2186 and 110-23678 IU/L, respectively) and even family 3, who received three doses (median = 271 IU/L, range = 263-305 IU/L).

A significant association between acquiring HBV and low ant-HBV titer was observed in our study (P= 0.005) (Table 7).

3.5 Evaluation of the Hepatitis B vaccination program

Data analysis regarding family members with anti-HBV levels more than 1000IU/L, revealed no significant difference between groups completed the vaccination dose schedules (3-doses) and those received one or two doses ($P > 0.05$). However median titers of anti-HBV were higher in the group receiving 3-doses (1843 IU/L) than in corresponding group receiving 1 or 2 doses (1455

IU/L). Moreover, variation in anti-HBV titers was also noticed as differences between the lowest and highest values of the range of the studied families (25-23678 for members who received 3 doses and 25 –16881 IU/L for members who received one or two doses) (Figure 3).

3.6 Follow up results

After 6 months of follow up, the study samples were re-tested for both HBsAg and anti-HBV. Three additional HBV infections were observed, one case belonged to family 8 and the other 2 cases were found in another two new families. On the other hand, HBsAg clearance was observed in four cases among those found to be HBsAg positive in the initial stage of the study.

Table 3. Demographic data for the study population

Variables	Number (%)
Age-Group,Years	
< 10	53 (32.9%)
11-20	56 (34.8%)
21-30	40 (24.8%)
> 31	12 (7.4%)
Gender	
Males	90(55.9%)
Females	71(44.1%)
Place of residence	
Town	38 (23.6%)
Village	92 (57.1%)
Camp	31 (19.3%)
Received doses	
1	3 (1.8%)
2	50 (31.1%)
3	108 (67.1%)
Anti - HBV titer	
<1000 unit	103 (64%)
>1000 unit	58 (36%)

Table 4. Distribution of HBV-infected patients according to their age-groups

Age	HBsAg + ve No.(%)	HBsAg – ve No.(%)	Total	*P* value
< 10	1 (1.9%)	52 (98.1%)	53	NS
11-20	5 (8.9%)	51 (91.1%)	56	
21-30	7 (17.5%)	33 (82.5%)	40	
> 31	0 (00%)	12 (100%)	12	
Total	13 (8.1%)	148 (91.9%)	161	

NS, not statisticallly significant (*P>0.05)*

Table 5. Distribution of HBV-infected patients according to residence

Residence	HBsAg + ve No.(%)	HBsAg - ve No.(%)	Total	*P* value
Village	11 (12%)	81 (88%)	92	NS
Camp	2 (6.5%)	29 (93.5%)	31	
Town	00 (0.00%)	38 (100%)	38	
Total	13	148	161	

Table 6. Demographic and serological data of the 13 HBV-infected patients and their families

Family No.	No. of persons/ family	Anti-HBV range (IU/L)	Anti-HBV median (IU/L)	No. of doses	No. of HBV +	Anti-HBV titer of HBV + (IU/L)
1	8	85 – 8508	3123	3	1	85
2	12	25 – 1441	574	3	1	25
3	2	263 – 305	271	3	2	263, 305
4	6	212 – 1271	657	3	1	212
5	9	59 – 8695	1766	3	3	59, 203, 229
6	2	161 – 186	174	3	1	186
7	3	127 – 2186	1068	2	1	127
8	3	93 – 136	110	2	2	93, 136
9	2	110 – 23678	11894	2	1	110

Table 7. Association between anti-HBV titer and HBV infection

Anti-HBV titer (IU/L)	HBsAg + ve No.(%)	HBsAg - ve No.(%)	Total	*P* value
				0.005
>1000	0 (0.00%)	58 (100%)	58	
<1000	13 (12.6%)	91 (88.4%)	103	
Total	13	148	161	

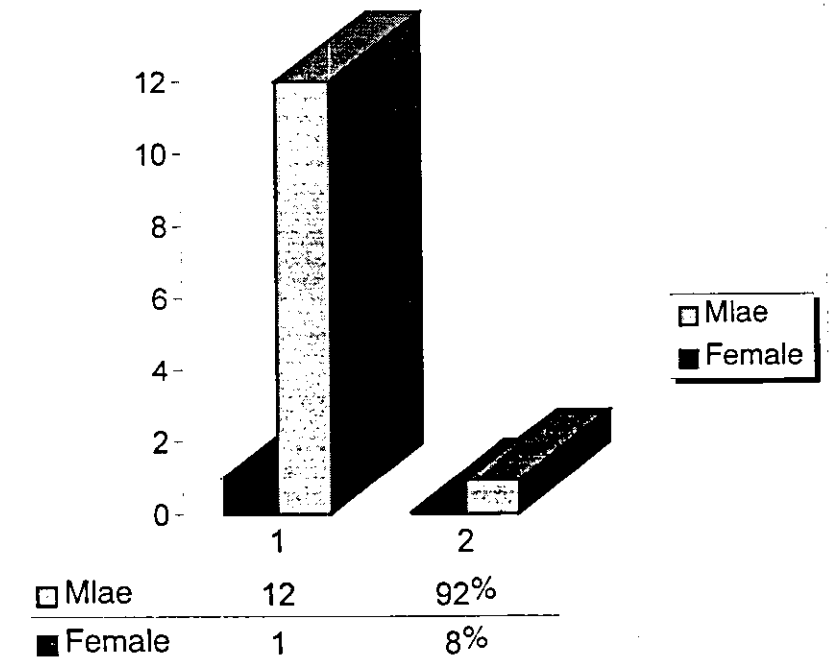

Figure 2. sex distribution of HBV infection

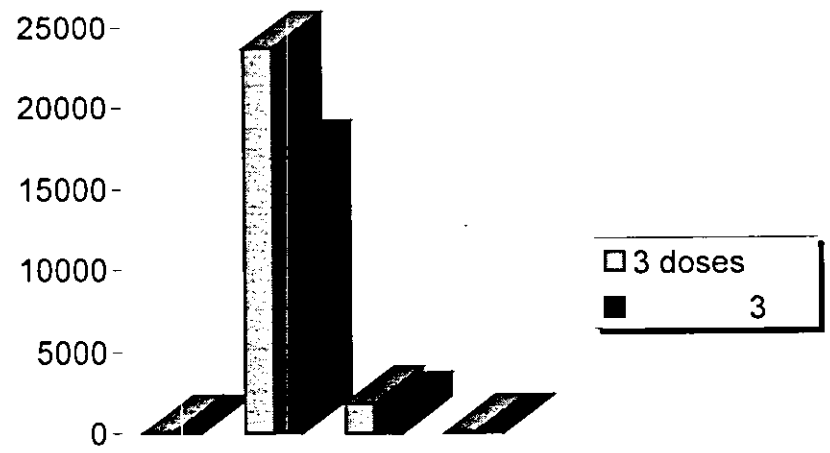

Figure 3. Association of anti-HBV and vaccination dose schedule. 1, Lowest value of anti-HBV IU/L; 2, Highest value IU/L; 3, Median value IU/L; 4, No. of persons with a protective anti-HBV titer i.e., more than 1000 IU/L

Chapter IV

Discussion and Concluding Remarks

Uninfected individuals living in close proximity with an HBV carrier are at greater risk of contracting HBV infection [40-42]. This is mainly due to the fact that HBV can survive even on dry surfaces for over a week.[10] It was also found to be transmitted through body fluids including urine, saliva/naso-pharyngeal fluids, semen, and menstrual fluids.[8, 9] However, it should be remembered that for HBV to infect, it still must gain entry into the bloodstream of an uninfected individual.

Over the last two decades the CDC recommended that all household contacts to HBV positive carrier should be immunized for hepatitis.[43] Three HB vaccines have been commercially marketed and available. The original Heptavax B®, a plasma-derived product, was introduced in the international market in 1982. The other two preparations are recombinant vaccines prepared from yeast cultures (*Saccharomyces cerevisiae*) that have been genetically altered to produce the hepatitis B surface antigen. Recombivax HB has been shown to induce protective antibody synthesis in more than 99% of healthy adult's 20-39 years of age. Hepavax-Gene is another recombinant vaccine developed by Korean Green Gross Corporation, which provides protection for several years (used in our case). Other products are also available such as Engerix-B, which is developed by a Belgium firm.

The present study was designed to evaluate the status of HBV and the efficacy of the vaccination programme among a total of 161 high-risk family members of hepatitis B virus carriers who were subjected to the vaccination programme in Tulkarm, Northern Palestine, 1997-1998.

Data presented in Table 3 shows that only 67.1% of the studied family members completed the vaccination dose schedules (3 doses given at 0, 1, and 6 months) and the remaining members received either one or two doses. Anti-HBV titer more than 1000 IU/L was achieved in 58 (36%) of the 161 family members. Our finding is an over estimate mainly due to the fact that around 33% of the study sample did not comply with the recommended vaccination dose schedule (3-doses of Hepavax-Gene). This is in contrast with the results of most of the studies on hepatitis B vaccination programmes which pointed that around 10% recipients of almost all HBV vaccines fail to make an adequate anti-HBV response and are classified as non-responders [43]. Taking into account the great proportion of expected non-responders in our study, 8.1% rate of positivity for HbsAg was observed among the study population.

It is worth noting here, that none of the vaccinated family members had post-test vaccination, which is usually performed one to six

months after the final injection, the only way for the detection of none responders. If anti-HBV have not developed, a fourth vaccine dose should be given with re-testing after one month. At the end of this schedule, a second three-injection regimen with the dose given the previous month considered as the initial dose should be given to those individuals who are still anti-HBV-negative.

If testing is delayed beyond six months, and particularly if it occurs years after completion of the series as in our case, interpretation of the results is much more difficult. A person testing negative at this time may be a non-responder or simply a responder who has lost detectable antibodies but is still protected from contracting the clinical disease as a result of their immunological memory.

Since protection is expected to lasts at least ten years due to the anamnestic response (based on the observation that no one receiving the original vaccine who was immuno-competent and responded has developed clinical hepatitis B) [44]. The finding of 13 (8.1%) HBsAg positive cases among vaccinated study group in our study is contradictory with previous reports. On the other hand, the issue of none responders and unrecognized infection (present at the time of vaccination / a symptomatic or long incubation period) may partially account for such high incidence rate of infection. One should also try to assess the duration of immunological

memory among such risk groups and or should look for an alternative vaccine that can overcome this non-responsiveness. An alternative approach for developing such vaccines was proposed by Pride, 1993 [45] were the production of anti-idiotypic vaccines are unique because they are not based on the conventional vaccine's need for antigenic in the form of the microbial agent responsible for the disease. Such vaccines were found to be effective in non-responder experimental mice [46].

Statistical analysis failed to demonstrate significant differences in HBV infection regarding the age. However in the study group, HBV infection was common in adolescents and young adults (Table 4). In previous studies, Recombivax HB had been effective to induce protective anti-HBV synthesis in more than 99% of healthy adults. Thus, the high involvement of these age groups, in whom the infection outcome is unpredictable and consequential disability is possible, clearly calls for attention to think about alternative vaccines or to increase the vaccination compliance rate by educating and improving the tracking, communication and coordination channels with individuals involved in the process.

Another interesting finding is that out of the 13 HBsAg positive cases, 92% were males and 8% were females. The difference in the prevalence rates between males and females were statistically

significant ($P = 0.008$) as shown in Figure 2. Intersex variations in HBV infection might be explained by behavioral differences betwwen the two sexes such as sharing of shaving razors and more exposure to injuries during work in males.

Although differences in the prevalence rates of HBV infection according to residence were not statistically significant ($P = 0.07$) residence in the village was clearly associated with higher rate of HBV infection (84.6%) compared to that in town and camp (Table 5). This might be explained by the fact that both town and camp residence have access to a much better health services than those living in urban areas. On the other hand education and awareness may also play an important role in this respect.

The 13 HBV-infected cases were sporadic cases distributed among nine different families. Out of the 13 cases only 2 were reported to show clinical symptoms and none of the rest seems to represent clinical symptoms of HBV. Such breakthrough HBV infections were reported among vaccinated subjects living in endemic areas or who live with family HBV chronic carriers family members [41, 42].

It is worth noting here, that all the 13 HBV-infected family members had anti-HBV titers below the protective level (range = 25–305 IU/L). Hoever, a wide range in variation of ant-HBV titers in response to vaccination between different families and even

between family members in the same family was observed and may exceeds 200 folds (Table 6). This variation could be attributed to the potency of the immune response which is influenced by the initial vaccination dose as well as some of these cases may be challenged by live HBV being at close contact with HBV chronic carrier subjects.

Some authors report on the higher proportion of infected members in large families. In our study, there were no remarkable differences in the HBV infection familial dissemination according to the size of family.

According to the study on the probable routes of infection transmission, home contacts was the only leading route of of HBV infection, revealed in the 13 cases. Nine (69.2%) of the infection transmission cases attributed to brother-brother relationship and 4 (30.8%) to father-children relationship. Our data analysis failed to answer the question why the brother-brother relationship was associated with a higher risk than the father-children relationship. However, frequent, close contact and the use of a common materials might contribute to this finding.

A significant association between acquiring HBV and low ant-HBV titer was observed in our study (P= 0.005) (Table 7). This is a well-known fact as most studies indicate that subjects with anti-

HBV titer below the protective level are at high risk of acquiring the infection [47, 48]. According to the the data presented in Table 6, a lower anti-HBV titer was associated with vaccination dose among members of family number 8. This is in contrast with the results of members of families number 7 and 9 and even family 3, who received three doses. One should point out that high anti-HBV titers such as those observed in some individuals (families 1, 9 and 5) might de due to exposure to live HBV antigens that might trigger memory cells among subjects with protective anti-HBV level. The data of Figure 3 point in the same direction. Analysis regarding family members with anti-HBV levels more than 1000IU/L, revealed no significant difference between groups completed the vaccination dose schedules (3-doses) and those received one or two doses ($P > 0.05$). However median titers of anti-HBV were higher in the group receiving 3-doses than in corresponding group receiving 1 or 2 doses.

During follow up study 6 months after the initiation study, three new sporadic HBV infetion was detected. Such finding again emphasizes the need to evaluate the hepatitis B vaccination programme in Palestine. It is also important to point out that vaccination compliance, post-test and storage conditions might be behind HBV incidence among family members of HBV chronic

carriers. The observation of low clearance rate of HBsAg 30.7% HBV-infected cases, age range 12-39 and mean 26.23years) is inconsistent with the results of most studies on HBV clearance in adulthood stage which is 95% or higher [49, 50]. Thus, this finding of low clearance rate is another impact and emphasizes that cases with non-protective anti-HBV level and with close contact with HBV carriers is a real threat for re-infection.

Recommendations and Concluding Remarks

1. Post vaccination testing for anti-HBV is essential for the detection of none responders.

2. Periodic monitoring of anti-HBV levels with booster vaccinations is essential to maintain protective antibody levels among risk groups in general.

3. Increase the vaccination compliance rate by educating and improving the tracking, communication and coordination channels with those individuals involved in the process and by increasing staff resources.

4. Due to the fact that vaccination programmes specifically directed at groups with increased risk may end in failure as in our case, mass vaccination of children and / or adolescents must be considered.

5. Searching for alternative vaccines such as idiotype specific vaccines might provide more effective and protective among risk groups.

6. Compliance with vaccination dose, storage conditions and schedules is essential to assure the best efficacy of such vaccination programs.

7. Health educations program for the community and health workers

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الملخص

لقد صممت الدراسة الحالية بهدف تقييم الوضع الصحي والمتعلق بالتهاب الكبد الوبائي الفيروسي من نوع B وكذلك مدى نجاعة برنامج التطعيم الحكومي ضد هذا المرض عند الأشخاص المخالطين لأشخاص مصابون إصابة مزمنة وذلك في منطقة طولكرم – شمال فلسطين. لقد شملت هذه الدراسة ١٦١ مخالطا كانوا قد اخضعوا لبرنامج التطعيم الوقائي الحكومي وذلك في الفترة الزمنية ١٩٩٧-١٩٩٨.

لقد بينت الدراسة وجود نسبة إصابة تعادل ٨،١% (١٣ حالة) لدى المخالطين وكانت أعلى نسبة إصابة لدى الفئة العمرية ٢١-٣٠ حيث كانت نسبة الإصابة هي ٧،٥% ولم تكن الفرو قات في نسبة الإصابة ذات قيم دالة إحصائيا بين الفئات العمرية المختلفة.

لقد لوحظ كذلك فروقات ذات دلالة إحصائية في نسبة الإصابة المتعلقة بالجنس حيث كانت نسبة الإصابة لدى الذكور(٩٢%) أعلى منها عند الإناث (P= 0.008). وبالرغم من عدم وجود فروقات ذات دلالة إحصائية في نسبة الإصابة المتعلقة بمكان السكن إلا انه لوحظ أن نسبة الإصابة لدى المخالطين المقيمين في القرى هي أعلى بكثير مما هو ملاحظ لدى المقيمين في كل من المدينة والمخيمات.

ولقد لوحظ كذلك بان انتقال العدوى في تسع حالات ٦٩،٢% كان بين المخالطين من الاخوة أما الحالات الأربعة الأخرى فكانت بين الآباء والأبناء. ولم تلاحظ فروقات كبرى في الإصابة بين كلا من العائلات صغيرة وكبيرة الحجم.

أما فيما يتعلق بتركيز الاجسام المضادة بعد التطعيم لدى الأشخاص المخالطين المصابين فكانت اقل من الحد الواقي والمعروف عالميا (١٠٠٠ او اكثر وحدة دولية لكل لتر) ولم يلاحظ وجود فروقات ذات قيم دالة إحصائيا فيما يتعلق بعدد الجرعات التطعيمية وتركيز المضادات الحيوية (جرعات ١،٢،٣).

وفي دراسة لاحقة أجريت بعد فترة ستة اشهر من الدراسة الأولية لوحظ ظهور الإصابة لدى ثلاثة حالات أخرى وقد لوحظ كذلك اختفاء الإصابة لدى أربعة حالات من حالات الإصابة التي تم الكشف عنها في بداية الدراسة.