THE ROLE OF HEPATITIS B CORE AS SCREENING TEST

MOHD ANUAR BIN ISMAIL

SCHOOL OF HEALTH SCIENCES

UNIVERSITY SAINS MALAYSIA

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By

MOHD ANUAR BIN ISMAIL

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Abstract

Qualitative detection of antibody to hepatitis B virus core antigen (anti-HBc) is an important aid in diagnosis of acute, chronic, or resolved HBV infection in conjunction with other lab results and clinical information. A multi-center prospective study was conducted to validate the performance of the AxSYM CORE 2.0 assay in a diagnostic population by evaluating precision and performing method comparison. AxSYM CORE 2.0 is a competitive microparticle enzyme immunoassay (MEIA) for use on the AxSYM system for the qualitative detection of anti-HBc in human adult and pediatric serum or plasma.

A multi-center prospective study was conducted to validate the performance of the HbsAg and HbsAg Confirmatory assays in a diagnostic population. Assay performance was measured by evaluating precision and performing method comparison. Qualitative detection and confirmation of hepatitis B surface antigen (HbsAg) is an important aid in diagnosis of HBV infection. The HbsAg and HbsAg Confirmatory assays are chemiluminescent microparticle immunoassays for the qualitative detection and confirmation of HbsAg in human adult.

There are about 350 million chronic hepatitis B virus (HBV) carriers worldwide. A proactive approach to the management of this disease is likely to reduce the morbidity and mortality caused by HBV. This study aimed to approach the HB core test as a screening tool in blood bank. The test is designed to rapidly and accurately detect both the HBV surface antigen (HBsAg) and the HBV e antigen (HB Core). A cohort of 316 subjects was tested. The serum clinical sensitivity of the hepatitis B sAg or Hb core test was 99.75 and 96.37% for HBsAg and HB Core, respectively. Serum clinical specificity

was 99.32% for HBsAg and 98.99% for HB core. Analytical sensitivity was satisfactory for the purposes of population screening. Visual evaluation showed that the test signals were stable for at least 3 h after the recommended evaluation time. No interference or cross-reactivity was observed with known interfering substances and virologic markers. These results indicate that the hepatitis B sAg/ core test is well suited to the accurate detection of HBV carriers. In addition to the good clinical specificity and sensitivity of this test, its stability and user-friendly design mean that a correct performance, even under field conditions, is highly likely. Consequently, the hepatitis B sAg/core test has the potential to identify subjects who require HBV vaccination ($HBsAg^-$ and $HBeAg^-$) and HBV-infected individuals who might benefit most from antiviral therapy ($HBsAg^+$ and $HBeAg^+$).

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1. Introduction

1.1 Hepatitis B

Hepatitis B is one of the three most common types of viral hepatitis, along with hepatitis A and C. The Centers for Disease Control and Prevention (CDC) estimates that some 150,000-300,000 people in the U.S. are newly infected with the hepatitis B virus (HBV) each year, and that over 1 million people currently carry the virus. HBV incidence in the U.S. has decreased since 1982, when an effective vaccine became available. Chronic hepatitis B – like hepatitis C – can cause serious long-term liver damage. However, most people who are infected with HBV recover completely, and most chronic HBV carriers live healthy, mostly symptom-free lives.

1.2 Hepatitis B virus in blood bank

The risk of transfusion-transmitted hepatitis B virus (HBV) infection has been reduced by screening all blood donations for HBV surface antigen (HBsAg) since 1970. It was generally accepted that the disappearance of HBsAg indicates the clearance of HBV. The safety of blood products is one of the major issues in the area of transfusion medicine. Transmission of hepatitis B virus (HBV) infection through donated blood is more common than hepatitis C virus (HCV) infection (1:60000 vs. 1:103000) . In spite of availability of sensitive screening assay for detection of hepatitis B virus surface antigen (HBsAg), occasional cases of post-transfusion hepatitis B virus infection (PTH) are common. There are three possible explanations for false negative results in commercial assays. Blood donors infected with HBsAg mutants and those circulating low level of

viral protein may escape detection by screening assay and therefore, may affect the safety of blood supply.

Another explanation is that virus variants yield sequences that are not recognized by the antibodies employed in the assays. There are variants in other parts of the genome that down regulate the production of HBsAg. Occasionally, a superinfection with hepatitis C virus (HCV) may induce clearance of hepatitis B. This could be due to the dominant role of HCV in eliciting an immune response. Antibodies to hepatitis B core (HBc) antigen are marker of acute, chronic, or resolved HBV infection and remain detectable for life. These can be present in the absence of both HBsAg and anti-HBs antibodies, during the convalescent period following acute hepatitis B before the appearance of anti-HBs antibodies, or in patients who resolved infection but lost detectable anti-HBs antibodies. Anti-HBc is therefore detected in anyone who has been infected with HBV.

It has been demonstrated that some HBsAg negative individuals and those positives for anti-HBc continue to replicate HBV. These findings suggest that recovery from acute hepatitis B virus infection may not result in complete virus elimination, but rather the immune system keeps the virus at a very low level. A positive correlation has been shown between anti-HBc titre and detection of HBV-DNA in serum samples of HBsAg negative individual.

Currently, a number of countries, including United States screen all donations for anti HBc, which is not mandatory in some other countries. However, detection of HBV-DNA by polymerase chain reaction (PCR) has the same significance as detection of HBsAg, and indicates current HBV infection. All blood donations in Iran are collected from healthy donors, and tested for HBsAg as a marker of transmissible HBV. Though these

measures have resulted in low rates of transmission by transfusion but have not eliminated it fully.

Despite the availability of an effective vaccine, HBV infection continues to be an important problem in Iran and nearly 8000 to 10000 deaths occur each year due to this sequelae. In Iran, the rate of asymptomatic hepatitis B carriers (HBsAg positive) varies between 0 and 3.9 per cent with an average of 1.7 per cent. The prevalence of HBV carriers in asymptomatic healthy blood donor in Fars province; the southwest of Iran was about 1 per cent. In a study on 4930 healthy blood donors in Hamadan province in Iran, 5.1 per cent were only positive for anti-HBc, without any detectable HBsAg, but the presence of HBV-DNA has not been determined in this study.

1.3 Transmission and Prevention

Hepatitis B (previously known as serum hepatitis) is caused by the hepatitis B virus (HBV); a member of the hepadnaviridae family that replicates (multiplies) in liver cells. Although the routes of transmission, symptoms, and long-term effects of hepatitis B and hepatitis C are similar in many respects, the diseases are caused by two distinct viruses that are not closely related.

Like the hepatitis C virus (HCV), HBV is a blood-borne virus. It can be transmitted by sharing drug paraphernalia that comes into contact with blood, including needles, syringes, and other injection equipment (e.g., cookers, cottons), cocaine straws, and crack pipes. Use only new needles; if you must share, carefully clean needles and syringes with bleach between users. New, disposable needles should be used for tattooing, piercing, and acupuncture. Do not share personal items such as toothbrushes, razors, or manicure tools.

Cover all open cuts, sores, and rashes. Properly dispose of bandages, menstrual pads, tampons, and other material that comes into contact with blood or body fluids. Healthcare workers should exercise universal precautions such as wearing gloves and facial protection when working with blood and body fluids.

Although many people previously contracted hepatitis through blood transfusions, donated blood has been tested for HBV since 1975 and for HCV since 1992. Transfusions are now considered quite safe. People do not get HBV or HCV from donating blood.

HBV is found in semen and vaginal fluid, and is more likely than HCV to be transmitted through sexual activity. The CDC estimates that 30-60% of new HBV infections may be sexually transmitted. Use condoms or latex barriers such as dental dams to reduce the chances of sexual transmission. Pregnant women can transmit HBV to their infants during birth. The risk of perinatal transmission varies widely depending on the mother's HBV antigen status and viral load. It is recommended that all pregnant women be tested for HBV; if they have the virus, their infants can be vaccinated and given anti-HBV antibodies at birth. Although HBV antigens are found in breast milk, there is no evidence that breast feeding transmits the virus.

HBV is present in saliva. However, hepatitis B is not known to be transmitted by sharing silverware or drinking glasses, through sneezing or coughing, or by kissing. There are no documented cases of HBV transmission via urine, feces, sweat, tears, or vomit. As with hepatitis A, hepatitis B is sometimes spread through household contact with a person who carries the virus. People who immigrated or were adopted from areas with a high revalence of hepatitis B (e.g., Asia, sub-Saharan Africa, and the Middle East) and those

who travel to these areas for extended periods of time are also at higher risk. In as many as 30-40% of cases, the route of HBV transmission is unknown.

HBV is a relatively hardy virus; studies suggests that it can live in dried blood for up to ten days and on some surfaces for as long as 30 days. Carefully clean and disinfect any surfaces that come into contact with blood and other body fluids; a solution of one part bleach to ten parts water effectively kills HBV.

1.4 Disease prevalence

The management of hepatitis B virus (HBV) infection presents us with many challenges. Disease prevention, as well as supervision of chronic carriers of the infection, remains a key concern. Despite a rapid increase in the Expanded Program on Immunization (EPI) coverage, the global disease burden remains high. It is estimated that around 2 billion individuals worldwide have evidence of present or past infection with HBV. Approximately 350 million are chronic carriers of the virus, and it is expected that between 15% and 25% of them will die as a result of HBV. Disease progression can take up to 30 years; therefore an estimated 1 million individuals will die each year from chronic hepatitis, cirrhosis, or hepatocellular carcinoma.

1.4.1 High prevalence

Globally, chronic hepatitis B (CHB) is widespread; approximately 45% of the global populations live in areas of high prevalence HBV. These areas include sub-Saharan Africa, aboriginal Australia, the East Mediterranean, South-east Asia (although Singapore, Taiwan and Malaysia are rapidly becoming areas of low/intermediate prevalence as a result of vaccination 1), South America, the Pacific Islands (excluding Japan), and the Inuit communities of Canada. It is expected that more than 8% of the population in these areas are positive to hepatitis B surface antigen (HBsAg). In high prevalence regions, the lifetime risk of HBV infection is greater than 60%, and most infections are acquired at birth or during early childhood when the risk of developing chronic infection is greatest. Because most infections in children are asymptomatic, very little acute disease related to HBV occurs, but rates of chronic liver disease and liver cancer in adults are very high.

1.4.2 Intermediate prevalence

Areas of intermediate prevalence include the countries that formed the old USSR, Eastern Europe, Japan, South-west Asia, Israel, and the Amazon Basin of South America. Approximately 43% of the global population live in areas of intermediate prevalence, with 2–7% of the population being hepatitis B surface antigen (HBsAg)-positive. The lifetime risk of being infected is 20–60% and infections occur in all age groups. Acute disease related to HBV is common because many infections occur in adolescents and adults; however, the high rates of chronic infection are maintained primarily by infections occurring in infants and children.

1.4.3 Low prevalence

Only 12% of the populations live in areas with low prevalence and less than 2% of this population is HBsAg-positive. In low prevalence areas (e.g. North America, Canada, Mexico, Western Europe, Australia, and New Zealand [with the exception of the Maori population]), the lifetime risk of infection is less than 20%. Most HBV infections in these areas occur in adults in relatively well-defined risk groups, including injecting drug users, homosexual men, and household contacts of HBV carriers.

1.5 Symptoms and Long-Term Effects

HBV has an incubation period of 40-180 days. Acute (early) symptoms may include fever, nausea, vomiting, abdominal pain, loss of appetite (anorexia), itching (pruritis), muscle and joint aches, and fatigue. Some people also develop jaundice (yellowish discoloration of the skin and sclera), which is a sign that the liver is not properly processing bilirubin, a pigment released when old red blood cells are broken down; this may also lead to dark-colored urine and pale-colored stools. The acute symptoms of hepatitis B tend to be more severe than those of hepatitis C.

However, 30-40% of people with acute hepatitis B have no symptoms. In a minority of cases, people with acute hepatitis B can develop severe or fatal fulminant liver inflammation; this is more likely in the elderly. Recovery from acute hepatitis usually takes 2-12 months, during this time a person may continue to experience fatigue and abdominal tenderness. Those who recover completely from hepatitis B are immune to HBV re-infection (although they can still contract hepatitis A and C).

Most people's immune systems clear HBV from their bodies, but some develop chronic hepatitis B (that is, the virus is still present after six months). HBV is much less likely than HCV to become chronic. Only 5-10% of people who are infected with HBV as adults develop chronic hepatitis B, compared to the 80-85% of HCV-infected people who develop chronic hepatitis C. The chances of developing chronic hepatitis B are much higher for infants or children; an estimated 80-90% of HBV-infected infants and 30-50% of HBV-infected children under age five develop chronic disease. Men are more likely than women to develop chronic hepatitis B.

Symptoms of chronic hepatitis B may include persistent or intermittent fatigue, nausea, anorexia, and abdominal pain. Chronic carriers of inactive HBV often have no symptoms and normal laboratory tests, but they can still transmit the virus to others.

Long-term effects of HBV infection are similar to those of chronic hepatitis C, including liver scarring (cirrhosis), fibrous deposits (fibrosis), primary liver cancer (hepatocellular carcinoma), and liver failure. It is estimated that 20-30% of people with chronic hepatitis B will go on to develop cirrhosis. Chronic hepatitis B tends to progress more rapidly than chronic hepatitis C. If liver damage progresses so far that the liver cannot carry out its essential functions, people may experience symptoms and related conditions including blood vessel damage, bleeding varicose veins in the esophagus or stomach, fluid in the abdomen (ascites), cognitive dysfunction, and coma. Severe liver failure may necessitate a liver transplant. HBV is responsible for estimated 5,000-6,000 deaths per year in the U.S.

1.6 Laboratory finding in HBV infection

- Hepatitis B surface antigen (HBsAg) -- this represents the first viral marker present in blood tests after the patient is infected. It usually disappears from the blood in 1-2 months.
- Hepatitis B core antibody (Anti-HBc) -- this is usually detected within 1-2 weeks of the appearance of hepatitis B surface antigen.
- Hepatitis B surface antibody (Anti-HBs) -- this is found both in those who have been immunized and those who have recovered from hepatitis infection.
- Both hepatitis B surface antibody and core antibody persist indefinitely in the blood of patients who have recovered from hepatitis B.
- Liver enzyme (transaminase) blood levels may be elevated due to liver damage.
- Albumin levels may be low and prothrombin time may be prolonged due to severe liver failure.

1.6.1 Hepatitis B virus (HBV) testing

HBV is transmitted through infected body fluids, including blood, semen, and vaginal fluids (including menstrual blood). It is also can be transmitted from a pregnant woman to her child at or near the time of birth.

- Hepatitis B surface antigen (HBsAg) is one of the most frequently performed tests for HBV. This HBV antigen is the earliest indicator of an active hepatitis B infection. This antigen may be present before symptoms appear. If this antigen

level remains high for more than 6 months, then you will probably become a carrier of HBV, meaning you can transmit it to others throughout your life.

- Hepatitis B surface antibody (HBsAb) is also one of the most common tests for HBV. Usually this antibody appears about 4 weeks after HBsAg disappears. This antibody also protects you from getting HBV again in the future. The test is done to determine the need for vaccination; the antibody is present in the body after vaccination. Occasionally your test may show that you have both the HBsAg and HBsAb; in this case, you are still contagious.
- Hepatitis B core antigen (HBcAg). Currently, there is no test to find this antigen.
- Hepatitis B core antibody (HBcAb) is an antibody to the hepatitis B core antigen. This antibody appears about 1 month after an active HBV infection. It can be found in people who had an infection in the past and in those with long-term (chronic) HBV. It usually is present for life.
- Hepatitis B core antibody IgM (HBcAbIgM) is also an antibody to the hepatitis B core antigen. It shows a recent infection in the last 6 months.
- Hepatitis B e-antigen (HBeAg) is an HBV protein that is only present during an active HBV infection. This test determines how contagious you are. Testing for this antigen can also be used to monitor the effectiveness of treatment for HBV.
- Hepatitis B e-antibody (HBeAb) shows that the active stage of the HBV infection is almost over and your risk of being contagious is greatly reduced. HBeAb is usually present during chronic HBV infections.

HBV DNA testing finds genetic material (DNA) from the hepatitis B virus. Currently, quantitative HBV DNA tests are done. A high HBV DNA level means that the virus is

multiplying in your body and you are very contagious. If you have a chronic HBV infection, an elevated viral DNA level means you are at an increased risk for chronic hepatitis and may be consider a treatment. Testing for HBV DNA also is important to monitor the effectiveness of treatment for chronic HBV infection. HBV DNA testing is a more sensitive test than HBeAg (above) for detecting HBV in the blood.

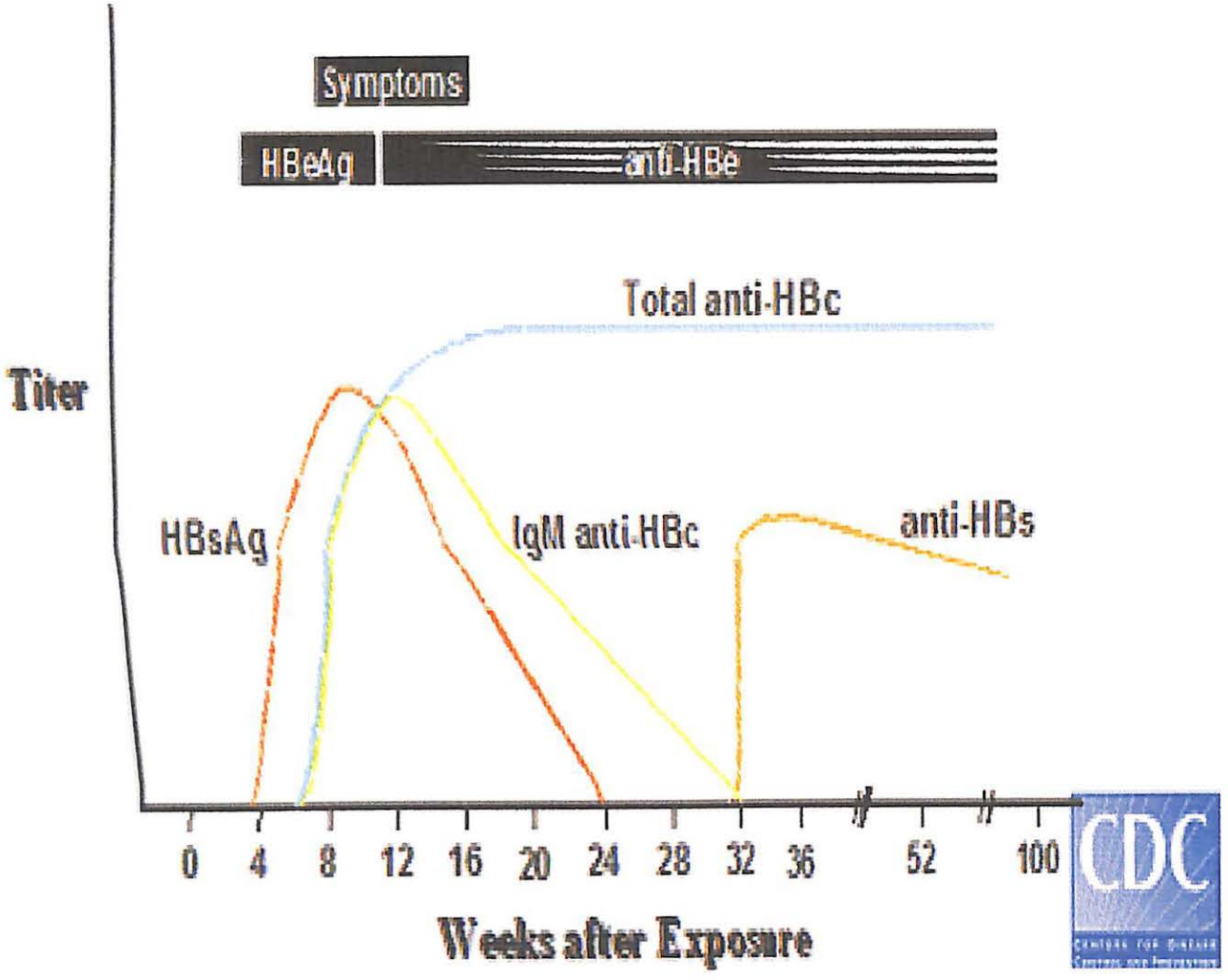

Figure 1: Acute Hepatitis B Virus Infection with Recovery Typical Serological Course

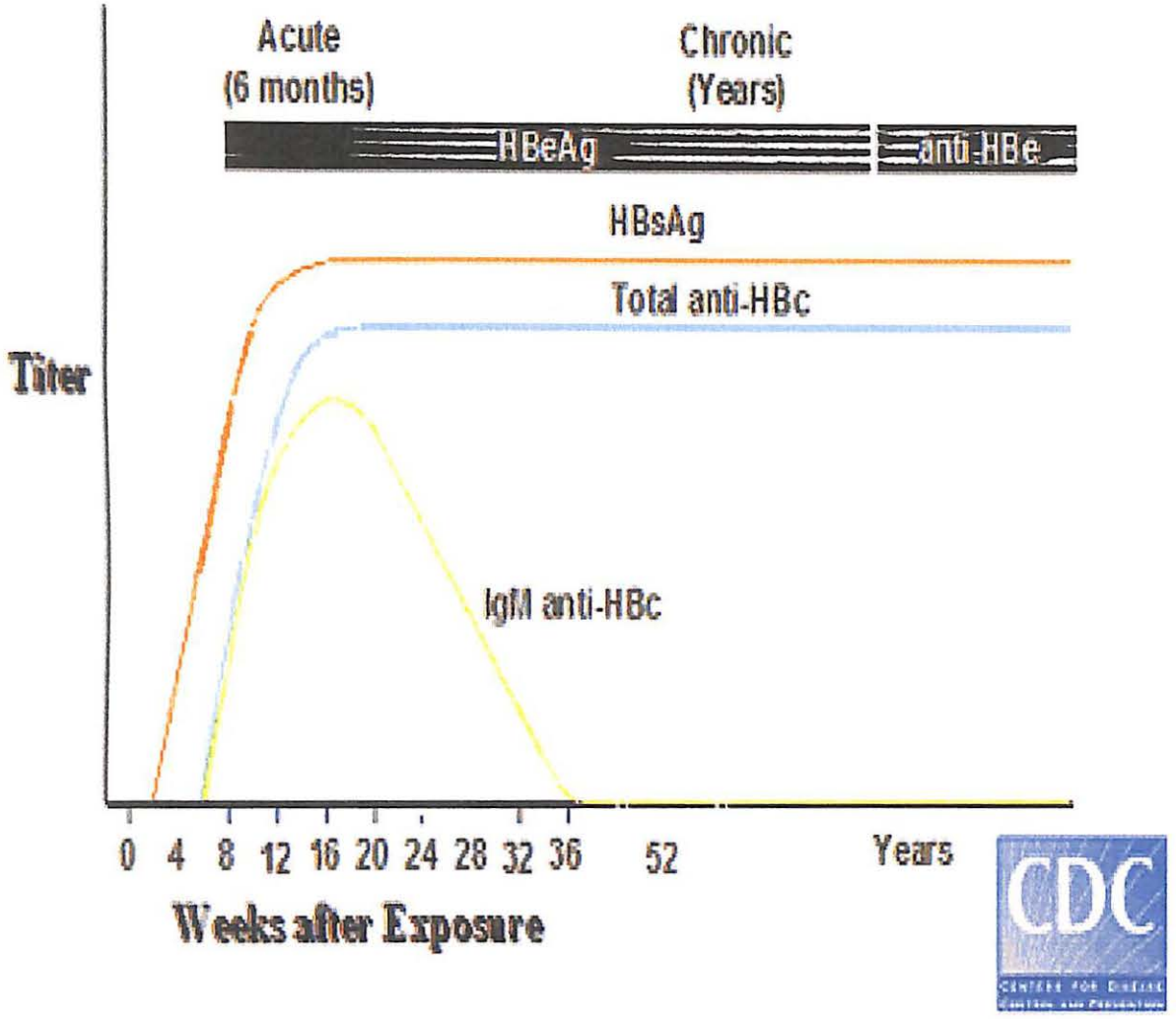

Figure 2: Progression to Chronic Hepatitis B Virus Infection Typical Serological Course

1.7 Diagnosis of Hepatitis

Hepatitis B is diagnosed and staged by looking at a complex combination of HBV antigens and antibodies in the blood. HBV has three antigens: HBsAg (surface), HBcAg (core), and HBeAg. The immune system produces three corresponding antibodies: anti-HBs, anti-HBc, and anti-HBe. The presence of HBsAg in the blood indicates that a person currently has hepatitis B—which may be either active or inactive (carrier state) – and can transmit the disease to others. The presence of HBeAg indicates that HBV is actively replicating and that a person is highly infectious and at greater risk for liver damage. The presence of anti-HBs antibodies in the absence of HBsAg shows that a person was previously infected with HBV but has recovered; they no longer have active disease, cannot transmit the virus, and are now immune (most people who have received the HBV vaccine have anti-HBs antibodies).

Today there are tests that directly measure HBV DNA (genetic material) rather than antigens and antibodies. Two common tests are the polymerase chain reaction (PCR) and branched DNA (bDNA) assays; others are being developed. A detectable viral load indicates that the virus is actively replicating; asymptomatic HBV carriers may shown little or no evidence of HBV replication. In addition to determining the presence of HBV, viral load tests are also used to monitor how well treatment is working.

1.8 HBV Coinfection

Because HBV and HCV are transmitted by similar means, some people (especially injection drug users) are infected with both viruses. Coinfection refers to simultaneous infection with HBV and HCV, while super infection refers to a person getting the second virus after they have already been infected with the first one. Research indicates that coinfection and super infection can lead to more serious liver damage than infection with either HBV or HCV alone. Affected people are at higher risk for liver cancer and for fulminant hepatitis, a serious acute inflammation that can result in rapid liver damage. Some studies suggest that even people who have recovered from hepatitis B may be more likely to develop cirrhosis and primary liver cancer if they later contract HCV. Because dual infection can lead to more severe liver disease, people with HCV should ask their doctor about getting vaccinated against hepatitis B and hepatitis A.

1.9 Vaccination

Unlike hepatitis C, hepatitis B can be prevented with a vaccine. There are currently two HBV vaccines, SmithKline Beecham's Energix-B and Merck's Recombivax HB. The vaccine is typically administered as a series of three intramuscular injections given over six months (the second injection one month after the first, and the third injection five months later). If the series is not completed within six months, it is necessary to start over. An alternate 2-dose vaccine schedule has been FDA-approved for adolescents aged 11-15. The HBV vaccine is now recommended as part of the standard childhood vaccination series, and for adolescents who were not vaccinated as children.

It is also recommended for (among others) injection drug users, healthcare workers, sexually active adults, and household contacts of HBV carriers. In May 2001, the FDA approved a new combined hepatitis A/hepatitis B vaccine (Twinrix).

If a person has recently been exposed to HBV, post exposure prophylaxis using immune globulin (injected antibodies) can help prevent a person from developing hepatitis B or reduce the length and severity of illness. This procedure, known as passive immunization, is effective about 75% of the time. Anti-HBV immune globulin (HBIG) should be given within 72 hours after exposure. HBIG can also help prevent the transmission of the HBV from a pregnant woman to her baby if given within 12 hours after birth. Because immune globulin only confers temporary protection (about three months), it is typically administered along with the first dose of the HBV vaccine.

1.10 Objectives

i. To measure the important of HB Core Ag as a screening tool.

ii. To detect HB Core Ag among blood donors.

iii. To compare specificity and sensitivity of tests to Hepatitis B virus.

iv. To identify the prevalence of hepatitis B among blood donors.