1. INTRODUCTION

Hepatitis B Virus (HBV) is a member of the hepadnaviridae family. It is spherical, partially double stranded, enveloped DNA virus, measures about 40-42 nm in diameter. The virus particle (Virion) consists of an outer lipid envelop, an icosahedral nucleocapsid encloses the viral DNA and DNA polymerase that have reverse transcriptase activity. Blumberg et al., (1965) first reported that the protein antigen in the serum of Australian patients known as Australian antigen which was a significant breakthrough in understanding of serum hepatitis. The first finding of hepadnaviruses in naturally infected woodchuck, tree squirrels and pekin ducks has provided animal model along with the availability of human hepatoma cell lines. Some of these viruses were discovered by screening of animals with the test designed for diagnosing hepatitis B in man (Blumberg et al., 1965).

1.1. Global Scenario of Hepatitis B virus

Hepatitis B virus (HBV) infection is an important global health problem. It is estimated that there are 350 million HBV carriers in the world. 2, 50,000 people die annually due to HBV related liver diseases. The global prevalence of HBV varies widely among HBV carriers, 75% are found in Asia. The high prevalence zones 7-20% are South East Asia, China and Sub-Sharan Africa. Low prevalence zone 0-2% are United states and Canada, Western Europe, Australia and New Zealand and intermediate zones 2-7% are Mediterranean countries, Japan, Central Asia, middle East and South America (Sita Naik , 2006).

1.2. Etiological agents of viral hepatitis

Viral hepatitis is a systematic infection primarily involving the liver. Therefore an inflammation of the liver caused by a number of etiological agents such as viruses, bacteria, fungi, parasites, drugs, and chemical are referred as hepatitis (Blumberg, 2002). Hepatitis was characterized by distortion of the normal hepatic lobular architecture due to varying degree of
necrosis of individual liver cells or group of liver cell i.e., acute (co-infection) or chronic (super infection) inflammation and Kupffer cell enlargement and proliferation (super infection). There is usually some degree of disruption of normal bile flow, which cause, jaundice. The severity of the disease is highly variable often unpredictable (Blumberg, 2002).

Hepatitis A, B, C, D E and G are the causative agent of viral hepatitis which causes inflammation of the liver namely chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC). Hepatitis B (HBV) has been a significant health problem worldwide particularly high incidence in South East Asia, China and Sub-Saharan Africa (Lee, 1997).

1.3. Transmission of Hepatitis B virus

HBV is transmitted through percutaneous and premucosal exposure to infective blood and body fluids by transfusion of blood or blood products, hemodialysis, use of contaminated needles, syringes and other sharp instruments, needle-stick injuries, oral surgery, prenatal exposure or sexual exposure. The heterosexual activity is associated for 27% of cases, whereas homosexual activity is for 11%, and injection drug abuser is for 14% (Mahon et al., 1985). Transmission of HBV in the United States and other developed countries primarily is through parenteral or sexual exposure, male homosexual activity, parenteral drug use, occupational exposure to blood, sexual or household exposure to an HBV-infected contact, and heterosexual activity with multiple partners (Alter, 1997).

1.3.1. Horizontal transmission of Hepatitis B virus

Horizontal transmission of virus by direct transmission person-to-person, from healthcare workers, sexual contact, parenteral exposure and indirectly by unsafe medical practice transfusions, haemodialysis and child to child called horizontal transmission is responsible for the majority of HBV infections and carriers. Universal infant or adolescent immunization is the proper strategy for long range control of hepatitis B infection everywhere in lower or
higher endemic areas. After the age of seven, children exhibit an adult pattern of disease outcome with about 5% to 10% becoming carriers. About 25% of children infected under seven will become carriers. The younger the child, more likely this will occur, with 30% to 50% of children infected between 1 to 4 years of age and up to 90% to 95% of infected infants becomes carriers. These carriers form a pool of infectious individuals who will later infect others in the community and eventually their own offspring (Anja and Meier, 2010).

1.3.2. Vertical transmission of Hepatitis B virus

Vertical transmission infects from mother to babies in perinatal period is an important mode of transmission in areas with high prevalence of HBV infection. The significance of HBV infection during pregnancy derives a major part from its potential to transmitted vertically (Okada et al., 1975). More than 90% of these women are chronic HBV carriers, although women acutely infected with the virus during pregnancy may also transmit to their children. HBeAg positive carrier mothers have 70-90% chance of infecting their newborn prenatally, and almost all of these infected newborn become chronic carriers. 20% of infants born from HBeAg negative mothers become infected during delivery. Perinatal (mother to child) transmission is one of the most efficient and serious modes of HBV transmission. Prevention of perinatal transmission has rightly been a priority from the WHO perspective.

1.4. Clinical Manifestation and viral load of Hepatitis B virus

The clinical manifestations of Hepatitis B infection range in severity from no symptoms to fully developed Hepatitis. Signs and symptoms of Hepatitis B may include fever, malaise, anorexia, nausea, and abdominal discomfort, followed within a few days by jaundice (Francis and Mahoney, 1999). The incubation period of hepatitis B averages 120 days. Symptoms such as malaise and anorexia may precede jaundice by 1-2 weeks. Acute Hepatitis B infection causes long-term infection in 30%-90% of persons infected as infants or children and in 6%-10% of
adolescents and adults. Chronic infection may lead to chronic liver disease, liver scarring (cirrhosis), and liver cancer. The disease has four clinical phases, a) incubation period – the incubation period ranges from 45 – 120 days. b) Pre-jaundice phase – this phase can vary from several days to more than a week, in general patients in this phase have a mild fever, loss of appetite, nausea and vomiting. c) Jaundice phase – this phase has dark golden brown urine, pale stool, and yellowing of skin and eyes. This phase generally occurs within 10 days of initial symptoms. d) Convalescent phase - during this phase it is important to determine if the patient is still highly contagious and is a carrier.

Complication of acute viral hepatitis include fulminate which is very severe, rapidly developing form of the disease and chronic hepatitis which is characterize by liver cell death and inflammation that lasts longer than six months (Francis and Mahoney, 1999). Since hepatitis B virus infection can remain clinically silent, it is important to have accurate methods to determine the presence of viral replication to monitor treatment outcomes and to identify changes in viral activity before they provoke clinical symptoms. The most rigorous way of determining viral replication is to measure circulating viral DNA. The threshold for risk of liver damage is around $10^4$ virions / ml. A surrogate measure of viral load is the presence of circulating hepatitis B virus e antigen; this method has been extensively used because it is inexpensive and relatively simple compared to DNA measurement. Loss of this antigen and the appearance of anti-HBe antibody (HBeAg seroconversion) are associated with a decrease in viral DNA titers to below 104 copies / ml and clinical remission. However, certain variants of the hepatitis B virus carry mutations in the pre-core region of the HBV genome encoding the HBeAg that prevent determination of serological status (Fabien Zoulim, 2004).
1.5. Laboratory Diagnosis of Hepatitis B virus

HBV-specific assays are routinely used for the assessment of disease activity in persistent infection, for monitoring therapeutic regimens with antiviral agents and most importantly, for evaluating the infection in a donor’s blood to prevent recipient’s contamination (Tsitsilonis et al., 2004). Diagnosis of HBV infection is established by the serological detection of HBV protein product antigens (Ags) as well as host-produced antibodies (Abs). Serological markers are key elements in diagnosing acute HBV infection and determining its possible evolution towards chronicity (Sablon and Shapiro, 2005). HBVAg and anti-HBVAb detection is often carried out simultaneously in the same serum or plasma specimens, using enzyme-linked immunosorbent assays (ELISAs), highly specific for HBsAg, anti-HBs, anti-HBc, and HBeAg or anti-HBe).

HBsAg is the primary diagnostic marker used for screening blood products in hospitals and health-care facilities. The envelope protein of HBV, HBsAg, is a transmembrane glycoprotein usually shed in large amounts in the serum of infected individuals, where it is found as spherical or filamentous particles with a diameter of 22 nm (Weber, 2001). HBsAg is considered to be the sentinel marker for the confirmation of acute infection. Its presence can be detected as early as 6 weeks after exposure, and should therefore be assessed when or more classical symptoms are observed. HBsAg is also the key marker in determining whether hepatitis B infection has become chronic (Sablon and Shapiro, 2005). Serological detection of HBsAg involves the use of either monoclonal or polyclonal anti-HBs bound to solid-phase or second labeled anti-HBs to detect the captured antigen. Among the many commercially licensed HBsAg assays offered, enzyme-linked immunosorbent assays are the most commonly used. Despite the high performance of third-generation enzyme-linked immunosorbent assay in the detection of HBsAg, a high incidence of false-negative results has been reported (Ismail, 2004). Antibodies to HBsAg indicate
recovery from infection and found in those immunized with HBV vaccines, but it may become undetectable in patients who have recovered fully from infection (Ismail, 2004; Kidd-Ljunggren et al., 2004). Individuals who have resolved their HBV infection usually demonstrate both anti-HBs and anti-HBc in their serum. Anti-HBs testing is useful for identifying HBV-susceptible individuals in pre- and post vaccination screening programmes, where absence of these antibodies is indicative of susceptibility to HBV infection. This could be an indication for vaccination against HBV (Ismail, 2004).

As the immune system begins to mount its response to infection, an initial rise - then decline of anti-HBc IgM is observed. By contrast, anti-HBc IgG rises but persists even after acute infection has resolved (Sablon and Shapiro, 2005). Antibody against hepatitis B core antigen (anti-HBc) is found in individuals who have experienced natural infection with HBV. Conventionally, the presence of anti-HBc in the absence of HBsAg is interpreted as evidence of a past HBV infection (Alhababi et al., 2003). Recently, there has been concern about a subgroup of individuals with the serological pattern of ‘anti-HBc only’ in which anti-HBc is the only detectable HBV marker in the absence of HBsAg or anti-HBs. It was reported that ‘anti-HBc only’ was found in 10-20% of all individuals with HBV markers in areas of low HBV endemicity and about 10% of these individuals had detectable DNA. Other studies, however, have found a higher HBV DNA prevalence of up to 40% (Alhababi et al., 2003). Molecular methods have demonstrated the presence of the virus in patients with anti-HBc alone with a frequency varying from 0 to 90% (Shih et al., 1990 and Wang et al., 1991). The significance of anti-HBc in the absence of the surface antigen is somewhat controversial such finding could represent 1) a situation where anti-HBs and anti-HBe being undetectable, loss of detectable anti-HBs, the patient is immunized; 2) a case where HBsAg levels are very low to be detected with routine assays; mutations at the antigenic ‘a’ determinant region of HBsAg or other regions of the surface gene and mutations in promoter and enhancer
sequences 3) false-Positivity or cross-reactivity of anti-HBc; and 4) an immunological window period, in which HBsAg is already undetectable and the surface antibody is not yet detectable (Kleinman et al., 1997 and Alhababi et al., 2003). Anti-HBc only’ has been described frequently among individuals infected with HIV or HCV. It is possible that co-infection with these viruses could lead to down regulation or interference of HBsAg production. The phenomenon of ‘anti-HBc only’ is not rare in diagnostic settings. The significance of this phenomenon is unknown and it is not clear how this serological profile should be interpreted, it is also unclear whether all individuals with such serological pattern need further molecular investigations (Alhababi et al., 2003).

HBeAg is considered as a better marker of viral blood infection, whereas the development of host Abs to HBe (anti-HBe) indicates the assessment of immunity and the reduction of viral replication in the infected individual (Sablon and Shapiro, 2005). Serum HBV DNA is indicative of active viral replication. It may be present at level exceeding $10^5$ to $10^6$ copies / ml and can be identified some 6-12 weeks after exposure to the virus or even earlier if PCR-based methods are used (Sablon and Shapiro, 2005). Over the past decade, improvements in molecular technology, permitting detection of as few as 10 copies/ml of HBV DNA in serum have led to redefinitions of chronic HBV infection, as well as thresholds for antiviral treatment (Servoss and Friedman, 2004). HBV DNA assays are not presently recommended for the routine evaluation and management of patients with chronic HBV infections, they nevertheless provide very useful adjunct information concerning viral replication – especially in situations when patient serological profiles fall outside of classical patterns (Kimura et al., 2003). Monitoring of serum HBV DNA level is a consistent method for the assessment of potency of antiviral therapy (Ismail, 2004). Several assays for the quantitative measurement of HBV DNA have been developed, such as the branched-chain DNA signal amplification assay and transcription mediated amplification (TMA)-based or
PCR-based nucleic acid amplification assays. Signal amplification assays have sensitivities approaching 1 pg of DNA ($10^5$-$10^6$ genome copies) or even to $10^3$ genome copies. Alternatively, HBV DNA detection based on a nested PCR approach can detect as few as $10^2$-$10^3$ genome copies. At such low titers, problems with contamination and reproducibility may lead to false-positive results. Commercial assays that make use of semi-automated systems can overcome these limitations. Attempts to grow HBV in standard cell lines have not been successful (Kidd-Ljunggren et al., 2004).

Currently, routine serological diagnosis relies on the detection of three pairs of antigens and antibodies: Hepatitis B surface antigen (HBsAg) and hepatitis Be antigen (HBeAg) and its corresponding antibody (anti-HBe). These are the primary markers for the diagnosis of routine hepatitis viral infection (Table – 1).

Table -1: Primary markers for the diagnosis of acute hepatitis B infection

(CDC- MMWR, 2004)

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<thead>
<tr>
<th>S.No</th>
<th>Diagnosis</th>
<th>Serological markers HBV Antigens</th>
<th>Anti-HBV Antibodies</th>
<th>Virological markers HBV DNA</th>
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<tr>
<td></td>
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<td>HBsAg</td>
<td>HBeAg</td>
<td>Anti-HBc</td>
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<td>1</td>
<td>Acute HBV</td>
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<td>5</td>
<td>Past Infection</td>
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1.6. Strategies and purpose for screening of pregnant women

The purpose of screening pregnant women is to identify those pregnancies where intervention would be of benefit to mother or the baby. In the context of hepatitis B virus (HBV) the appropriate intervention would be immune prophylaxis with hepatitis B vaccine with or without the addition of passive prophylaxis with specific hepatitis B hyper immune globulin (HBIG). Strategies for infant vaccination against hepatitis B are influenced by the prevalence of persistent infection in the community. Where the prevalence exceeds 2%, universal vaccination of infants is appropriate. In countries of very low prevalence (<0.5% HBsAg), a targeted approach may be preferred. Babies at high risk of acquiring HBV infection from the carrier mother would be identified through antenatal screening.

Various strategies for antenatal screening have been adopted universal, selective screening based on risk assessment, low prevalence countries, and screening pools of sera. Selective screening requires identification of mother at higher risk of infection. Distribution of participants according to age group, Educational status, Occupational exposure, Jaundice, Blood transfusion, Immunization status, History of Dental therapy, History of Surgery, History of intravenous drug use, Contact of known carrier, Hemodialysis, History of STD and Ethnic origin (Elizabeth Boxall, 1998).

1.7. Treatment of Hepatitis B virus

Treatment of acute infection is largely supportive and antiviral therapy is not indicated. The Primary goal of treatment is prevention of the complications of liver disease. A secondary goal is to decrease the number of chronic carriers who serve as a reservoir for HBV transmission. Because complication may take many years to develop, most treatment trials in patients with chronic HBV infections have used intermediate end points, such as inhibition of viral replication and improvement in liver histology, to evaluate efficacy. Loss
of HBV DNA and seroconversion from HBeAg to Anti-HBe are usually associated with normalization of liver enzymes and decreasing inflammation on liver biopsy. The goals of the therapy in patients with HBV infection are a reduction in the level of viremia and amelioration of hepatic dysfunction. Most of the studies have focused on chronically infected patients with elevated aminotransferase level and circulating HBeAg. There are clear indications for therapy in HBeAg-positive patients. They have an increased risk of early progression to chronic active hepatitis and cirrhosis and they have a high risk of hepatocellular carcinoma that is substantially higher than that for other carriers (Yang et al., 2002). By contrast, HBeAg-negative chronic carriers with viral loads below 10^5 genomes per milliliter and normal alanine aminotransferase values tend to have relatively stable course, with low rate of clinical or pathological progression (Franchis et al., 1993). At present, therapy is usually not offered to such patients. Some of the HBeAg-negative patients have liver dysfunction and substantial viremia (>10^5 molecules / milliliter). Results of recent trial suggest that many of these patients would also benefit from antiviral therapy.

1.7.1. Interferon

For many years, administration of interferon alfa (5 million to 10 million units subcutaneously three times per week for at least three months) was the main stay of therapy. About 30 percent of the patients who tolerated this regimen had a successful response, defined as a loss of HBeAg, the development of anti-HBe antibodies and a decline in serum alanine amino transferase level (Wong et al., 1993). Interferon alfa treatment of chronic HBV infections in patients with cirrhosis has been reported to reduce the risk of hepatocellular carcinoma. However, the side effects of the therapy with interferon alfa (fever, myalgias, thrombocytopenia and depression) make it a difficult treatment for many patients. Moreover, in many patients a flare of liver injury occurs during administration of interferon alfa, often just before or during clearance of HBeAg. This phenomenon may reflect the
immunomodulatory activity of interferon alfa, which, in addition to impairing HBV replication, can also cause up-regulation of MHC class I antigens on hepatocytes and thereby augment the recognition of infected cells by cytotoxic T lymphocytes. However treatment with interferon alfa is generally contraindicated in very advanced liver disease, since in such cases flares may precipitate overt liver failure (Ganem Don et al., 2004).

1.7.2. Antiviral Drugs

In the past decade, therapy for HBV infection has been revolutionized by the advent of drugs that directly block replication of HBV genome. All these drugs are nucleoside or nucleotide analogue that selectively target the viral reverse transcriptase. The first successful drug, Lamivudine emerged from screening for inhibitors of HIV reverse transcriptase and was introduced into clinical practice for management of HIV. Carriers of HIV who were also infected with HBV had substantial decline in HBV viremia when treated with lamivudine and such declines were also observed in patients with chronic hepatitis B who did not have HIV. In general treatment with lamivudine results in reduction of 3 to 4 logs in circulating levels of HBV DNA in the first three months of therapy, this decline is associated with more rapid loss of HBeAg, seroconversion to anti-HBe-positive status and improvement in serum aminotransferase levels. This drug is usually well tolerated, a factor that has led to the rapid displacement of interferon alfa from the roster of first-line therapies for HBV. Lamivudine is not an immunomodulator and can be used with decompensated cirrhosis (Villenvue et al., 2000). The principal limitation of Lamivudine mono therapy is the development of drug resistance, which is mediated largely by point mutation at YMDD motif at the catalytic center of the viral reverse transcriptase. By the end of one year therapy, 15 to 20 percent of the patients have resistant variants in the circulation; the figure rises 40 percent by two years and 67 percent by the fourth year (Liaw et al., 2002). Recently Food and Drug Administration (FDA) in the USA approved second antiviral drug, adefovir, to treat HBV infection. Adefovir
nucleotide analogue is a prodrug that undergoes two intracellular phosphorylations to yield the active drug, an inhibitor of viral polymerase. Initially developed as an inhibitor of HIV reverse transcriptase, it proved nephrotoxic in doses that were required for effective inhibition of HIV replication. However, in lower doses it has shown little nephrotoxicity and retains good efficacy against HBV in HBeAg positive patients, with a reduction of 3 to 4 log in viremia; the frequency of HBeAg seroconversion is enhanced, and there is histological improvement in the liver (Macellin et al., 2003). Moreover, the drug effectively inhibits the replication of lamivudine-resistant HBV mutants, both in vitro and in vivo (Ying et al., 2000).

1.8. Hepatitis B virus Vaccine and Immunophrophylaxis

The main strategies for the prevention of HBV infections are behavior modification to prevent disease transmission, i.e., safe sex practice, screening measures of blood and blood products, passive immunoprophylaxis and active immunization. No cure is available for hepatitis B, so prevention is crucial. Vaccines can provide protection in 90% to 95% of healthy persons. The vaccine can be given safely to infants, children, and adults in three doses over a period of 6 months.

1.8.1. Passive Immunoprophylaxis

Current recommendation for unvaccinated persons sustaining an exposure to HBV, post exposure prophylaxis with combination of HBIG (hepatitis B immunoglobulin) for rapid achievement of high titer circulating anti-HBs) and hepatitis B vaccine (for achievement of long-lasting immunity as well as its apparent efficacy in attenuating clinical illness after exposure) is recommended. For prenatal exposure of infants born to HBsAg-positive mothers, a single dose of HBIG, 0.5ml, should be administered intramuscularly in the thigh immediately after birth, followed by a complete course of three injections of recombinant
hepatitis B vaccine to be started within first 12 hours of life. For those experiencing a direct percutaneous inoculation or intra mucosal exposure to HBsAg positive blood or body fluid (e.g. accidental needle stick, other mucosal penetration, or ingestion), a single intramuscular dose of HBIG .0.06ml/kg, administer as soon after exposure as possible is followed by a complete course of hepatitis vaccine to begin within first week. For those exposed by sexual contact to a patient with acute hepatitis B, a single intra muscular dose of HBIG, 0.06ml/kg, should be given within 14 days of exposure, to be followed by a complete course of hepatitis B vaccine. (CDC, Morbidity and Mortality Weekly Report, 2004).

1.8.2. Active Immunization

The hepatitis B vaccine became available in 1982. Plasma-derived vaccine was available first and consisted of antigen particles isolated and purified from plasma of infected individuals. Although this vaccine was highly effective, concern about transmission of other infection led to development of recombinant vaccine. Recombinant vaccines are made by incorporating the surface gene (S-genes) of HBV in to different expression vectors (e.g., yeast, E.coli or mammalian cell lines). The yeast derived vaccine is the most widely available. The standard regimen is three doses of vaccine (at 0, 1 and 6 months) at doses of 20 microgram in adults and 10 microgram in children. Recommendation is to administer the vaccine by intramuscular injection in deltoid muscle for adult and in lateral aspect of thigh in children (Jinlin Hou et al., 2005).

HBV vaccine is highly effective (Szmonness et al., 1982). Approximately 80-90% of immune competent vaccines retain protective levels of anti-HBs for at least 5 years, and 60-80% for 10years. Thereafter anti-HBs becomes undetectable, protection persists against clinical hepatitis B, hepatitis B surface antigenaemia and chronic HBV infection. Currently booster immunization is not recommended routinely, except in immunosuppressed persons
who have lost detectable anti-HBs or immunocompetent, persons who sustain percutaneous HBsAg-positive inoculation after losing detectable antibody. Specially, hemodialysis patients, annual anti-HBs testing are recommended after vaccination; booster doses are recommended when anti HBs levels fall below 10mIU/ml.

1.8.3. Routine infant vaccination

Universal infant immunization is now recognized as proper strategy for every country for long term control of HBV infection and its sequelae (cirrhosis and liver cancer). WHO recommends routine immunization of all infants as an integral part of national immunization schedules. In countries of high disease endemicity (hepatitis B surface antigen (HBsAg) prevalence 8% or more), routine infant hepatitis B vaccination can rapidly reduce transmission because most chronic infection are acquired as a result of spread either from mother to baby or from child to child in first year of life. In countries of intermediate hepatitis B viral endemicity (HBsAg prevalence 2%-7%) and low endemicity (HBsAg prevalence below 2%), routine infant hepatitis B vaccination is also the highest priority. This is because a high proportion of chronic infections are acquired during childhood in these countries and most infections acquired during childhood occur among children born to mothers who are not infected with hepatitis B virus. These infections would not be prevented by perinatal hepatitis B preventions services that screen women for HBsAg and provide post exposure immunization for infants of HBsAg-positive mothers. (www.who.int/vaccines-diseases/hepatitis_b.shtml)

1.9. Effectiveness of early detection

The early detection of HBsAg in pregnant women can prevent infection in the newborn. Controlled trials, a cohort study and multiple series have shown that hepatitis B vaccine alone and in combination with hepatitis B immunoglobulin (HIBG) is effective in
preventing the development of chronic HBV infection in infants born to HBsAg – positive mothers. Vaccine, in combination with a single dose of HBIG given within 12 hours of birth, is 75-95% efficacious in preventing chronic HBV infection, whereas vaccine alone efficacy of 65-96%. The range of efficacy overlap, the efficacy of hepatitis B vaccine in combination with HBIG was generally greater than that of vaccine alone in studies that directly compared the two strategies, with the difference reaching statistical significance in two studies.

In the past prenatal testing for HBsAg was recommended only for pregnant women at high risk of having acquired HBV infection. The test may be repeated in third trimester if the women is initially HBsAg – negative and engages in high risk behavior such as injection drug use or if exposure to hepatitis B virus during pregnancy is suspected. Infant born to HBsAg – positive mothers should receive hepatitis B immunoglobulin (HBIG) 0.5 ml intramuscularly within 12 hours of birth (Sehgal et al., 1992).

The hepatitis B surface antigen (HBsAg) is most frequently used to screen for the presence of this infection. It is the first detectable viral antigen to appear during infection. However, early in an infection, this antigen may not be present and it may be undetectable later in the infection as it is being cleared by the host. The infectious virion contains an inner "core particle" enclosing viral genome. The icosahedral core particle is made of 180 or 240 copies of core protein, alternatively known as hepatitis B core antigen or HBcAg. During this 'window' in which the host remains infected but is successfully clearing the virus, IgM antibodies to the hepatitis B core antigen (anti-HBc IgM) may be the only serological evidence of disease. Shortly after the appearance of the HBsAg, another antigen named as the hepatitis Be antigen (HBeAg) will appear. Traditionally, the presence of HBeAg in a host's serum is associated with much higher rates of viral replication and enhanced infectivity;

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however, variants of the hepatitis B virus do not produce the 'e' antigen, so this rule does not always hold true. During the natural course of an infection, the HBeAg may be cleared and antibodies to the 'e' antigen (anti-HBe) will arise immediately afterwards. This conversion is usually associated with a dramatic decline in viral replication. If the host is able to clear the infection, eventually the HBsAg will become undetectable and will be followed by IgG antibodies to the hepatitis B surface antigen and core antigen, (anti-HBs and anti HBe IgG). The time between the removal of the HBsAg and the appearance of anti-HBs is called the window period. A person negative for HBsAg but positive for anti-HBs have either cleared an infection or has been vaccinated previously.

1.10. Genotyping of Hepatitis B virus and Phylogenetic Analysis

Hepatitis B surface antigen gene of hepatitis B virus was used for finding the relationships between individuals in the same species (Population study), different species (Phylogenetic studies) and various isolates (within species) from different parts of the world (Phylogeographical study). Phylogeny is the study of evolutionary relationships; the evolutionary history inferred from phylogenetic analysis is usually depicted as branching, treelike diagrams that represent an estimated pedigree of the inherited relationship among molecules, microorganisms, plant or animal species (Andreas and Francis, 2001).

One of the powerful implications of descent with modification relationships in that even when looking across different lines of evidence (eg. behavioral, molecular, biochemical and morphological). However, looking across many lines of evidence should overcome the messiness of particular characters and make it possible to identify the evolutionary ‘Single’ that defines the true historical relationship between taxa. Bioinformatics tools are primarily used to construct the tree based on sequence data alone (Susan, 2006).
HBV genotypes differ by more than 8% using the sequence of its complete genome or 4% using the sequence of SHBs (Wong and Chan, 2005). Early works have found a correlation between HBV genotypes and serological subtypes. Eight genotypes of hepatitis B virus (A-H) are currently recognized, and sub genotypes have recently been described in four of these genotypes (A, B, C and F). The genotypes show a distinct geographical distribution between and even within regions and are proving to be an invaluable tool in tracing the molecular evolution and patterns and modes of spread of hepatitis B virus. Genotypes A and D are most frequently observed in Europe, Africa, and North America, while genotypes B and C are prevalent in Asia. Genotype E is restricted to West Africa and genotype F is found in Central and South America. Genotype G was identified in France, Germany and North America. Recently, genotype H has been described in Central America (Kramvis et al., 2005). Structural and functional differences between genotypes can influence the severity, course and likelihood of complications and response to treatment of hepatitis B virus infection and possibly vaccination against the virus (Schaefer, 2005).

HBV has a reported mutation rate of 10 times greater compared with other DNA viruses. These mutations can occur naturally as well as due to selective pressure from antiviral therapy. Unlike cellular polymerases, the HBV Pol is a reverse transcriptase that lacks proofreading function that would permit it to recognize incorrectly incorporated nucleotides. As a result, HBV populations exist in the host as heterogeneous mixtures known as quasi-species (Locarnini and Bartholomeusz, 2005; Locarnini, 2004). There are five clinically relevant HBV mutant types. A mutation at the 1896 nucleotide (precore /core region) processing the production of the HBeAg was identified first. The prevalence of this mutant virus varies among different areas (Pan and Zhang, 2005).
Hepatitis B virus HBsAg gene has become popular because research of HBV genotype during the past 15 years has expanded the knowledge of the biology of HBV and the mechanism of liver diseases associated with HBV infections. This study therefore set out to determine the prevalence of HBsAg in pregnant women. The present study was carried out with the following objectives:

- To study prevalence of Hepatitis B infection among pregnant women attending hospital for delivery and normal checkup.
- Screening for hepatitis B surface antigen by strip method (qualitative test).
- To detect HBsAg by ELISA (indication of early during the course of infection and marker for the primary diagnosis of HBV infection).
- To detect HBeAg by ELISA (Conformation of viral replication and chronic carrier conformation).
- To detect HBeAb by ELISA (Conformation of clinical sign of recovery from infection).
- Detection of Hepatitis B virus DNA through DNA extraction method & amplification of HBV DNA through Polymerase Chain Reaction (PCR).
- Genotyping of hepatitis B virus using gene sequence. (Genetic diversity of hepatitis B virus).