II  Review of Literature
1.1 Prevalence of HBV infection

The global prevalence of HBsAg varies greatly and countries can be defined as having a high, intermediate and low prevalence of HBV infection based on a prevalence of HBsAg carriers of ≥8%, 2 to 7 %, and <2% respectively (Weinbaum et al., 2008). In developed countries, the prevalence is higher among those who immigrated from high or intermediate prevalence countries and in those with high risk behaviors (Mast et al., 2005). The prevalence of chronic HBV infection varies greatly in different parts of the world and are differentiated on the basis of endemicity as

1.1.1 High Endemicity

Hepatitis B is highly endemic in developing regions with large population such as South East Asia, China, sub-Saharan Africa and the Amazon Basin, where at least 8% of the population have chronic HBV infection. In these areas, 70–95% of the population shows past or present serological evidence of HBV infection (Heathcote et al., 2008). Since most infections in children are asymptomatic, there is little evidence of acute disease related to HBV, but the rates of chronic liver disease and liver cancer in adults are high (Alter et al., 2003).

1.1.2 Intermediate Endemicity

Hepatitis B is moderately endemic in part of Eastern and Southern Europe, the Middle East, India, Japan and part of South America. Between 10–60% of the population have evidence of infection, and 2-7 % has chronic infection. Acute disease related to HBV is common in these areas because many infections occur in adolescents and adults; however, the high rates of chronic infection are maintained mostly by infections occurring in infants and children. In India, HBsAg prevalence among the general population ranges from 2% to 8%, placing India in intermediate HBV endemicity zone
and the number of HBV carriers is estimated to be 50 million, forming the second largest global pool of chronic HBV infections (Datta et al., 2008).

1.1.3 Low Endemicity

The endemicity of HBV is low in most developed areas, such as North America, Northern and Western Europe and Australia. In these regions, HBV infects 5–7% of the population, and only 0.3-2% of the population has chronic infection (Roman et al., 2009). In these areas, most HBV infections occur in adolescents and young adults in relatively well-defined high-risk groups, including injection drug user, homosexual males, healthcare workers and patients who require regular blood transfusion or hemodialysis.

1.2 HBV Transmission

Blood is the most important vehicle for transmission, but other body fluids have also been implicated. HBV can survive outside the body for prolonged periods (Bond et al., 1981). Because HBV is resistant to breakdown outside the body, it is easily transmitted through contact with infected bodily fluids (Lavanchy et al., 2004). HBV can be detected in blood and derivatives as well as in saliva, semen, vaginal secretion, exudates from cutaneous ulcer and menstrual blood of infected individuals (Scott et al., 1980). HBV is transmitted by perinatal, percutaneous and sexual exposure, as well as by close person to person contact presumably by open cuts and sores, especially among children in hyperendemic areas (Mast et al., 2005). Immunosuppressed persons are more likely to develop chronic HBV infection after acute infection (Horvath et al., 1994). There is no reliable evidence that airborne infections occur and feces are not a source of infection.
1.2.1 Perinatal Transmission

Transmission of HBV from infected mothers to their babies can occur during the perinatal period and appears to be the most important factor in determining the prevalence of the infection in high endemicity areas, particularly in China and Southeast Asia. The risk of perinatal HBV infection ranges from 10–40% in HBeAg negative mothers to 70–90% in HBeAg-positive mothers (Alter et al., 2003). There are three possible routes of transmission of HBV from infected mothers to infants: transplacental transmission of HBV in utero; natal transmission during delivery; or postnatal transmission during care or through breast milk. For neonates and children younger than one year who acquire HBV infection perinatally, the risk of the infection becoming chronic is 90% (Hyams et al., 1995).

1.2.2 Sexual Transmission

Sexual transmission of hepatitis B is a major source of infection in all areas of the world, especially in the low endemic areas, such as North America. Hepatitis B is considered to be a sexually transmitted disease (STD). For a long time, homosexual men have been considered to be at the highest risk of infection due to sexual contact (70% of homosexual men were infected after 5 years of sexual activity) (Alter et al., 2003). Factors associated with increased risk of HBV infection include number of sexual partners, history of sexual transmitted disease, and positive serology for syphilis. Sexual partners of injection drug users, prostitutes, and clients of prostitutes are at particularly high risk for infection (Alter et al., 1994).

1.2.3 Parenteral/Percutaneous Transmission

The parenteral mode of transmission includes injection drug use, transfusions and dialysis, acupuncture, working in a health-care setting, tattooing and household contact. Although the risk for transfusion-associated HBV infection has been greatly reduced
since the screening of blood for HBV markers and the exclusion of donors who engage in high-risk activities, transmission is still possible when the blood donors are asymptomatic carrier with HBsAg negative (Luo et al., 1993). Parenteral/percutaneous transmission can occur during surgery, after needle-stick injuries, intravenous drug use and following procedures such as ear piercing, tattooing, acupuncture, circumcision and scarification. The nosocomial spread of HBV infection in the hospital, particularly in dialysis units, as well as in dental units, has been well described (Motta et al., 2009), even when infection control practices are followed.

1.3 Natural History

Chronic hepatitis B (CHB) infection is a dynamic process with replicative and a low replicative phases based on virus-host interaction. The natural history of CHB can be schematically divided into five phases, which are not necessarily sequential.

1.3.1 The “immune tolerant” phase is characterized by HBeAg positivity, high levels of HBV replication, normal or low levels of aminotransferases, mild or no liver necroinflammation and no or slow progression of fibrosis (Hoofnag1e et al., 2007). During this phase, the patients have no symptoms. Because of high levels of viremia, these patients are highly contagious.

1.3.2 The “immune reactive” phase is characterized by HBeAg positivity, a lower level of replication, increased or fluctuating levels of aminotransferases, moderate or severe liver necroinflammation and more rapid progression of fibrosis compared to the previous phase (Lok et al., 2007). It may last for several weeks to several years. This phase may occur after several years of immune tolerance and is more frequently reached in subjects infected during adulthood (Hoofnagle et al., 2007).

1.3.3 The “inactive HBV carrier state” may follow seroconversion from HBeAg to anti-HBe antibodies. This phase is usually preceded by a marked reduction of serum
HBV DNA levels followed by normalization of ALT levels and resolution of liver necroinflammation (Lok ASF et al., 2001). HBsAg loss and seroconversion to anti-HBs antibodies may occur spontaneously in 1–3% of cases per year, usually after several years with persistently undetectable HBV DNA (Martinot-Peignoux, 2002). The inactive carrier state may persist for a lifetime.

1.3.4 "HBeAg-negative CHB" may follow seroconversion from HBeAg to anti-HBe antibodies during the immune reactive phase and represents a later phase in the natural history of CHB. It is characterized by periodic reactivation with a pattern of fluctuating levels of HBV DNA and aminotransferases and active hepatitis. These patients are HBeAg negative and harbour HBV variants with nucleotide substitutions in the precore and/or the basal core promoter regions unable to express or expressing low levels of HBeAg. In the "HBsAg-negative phase" after HBsAg loss, low-level HBV replication may persist with detectable HBV DNA in the liver (Raimondo et al, 2008). Generally, HBV DNA is not detectable in the serum while anti- HBe antibodies with or without anti-HBs are detectable.

1.3.1.0 Factors influencing the natural history of chronic hepatitis B.

1.3.1.1 Age at Infection

The age at acquisition of hepatitis B infection influences the risk of chronicity, with rates of persistent infection being substantially higher in individuals infected perinatally or during infancy than in those infected as adults. Spontaneous HBeAg seroconversion rates are low in children and those infected early in life (Chang et al., 2000). During the immune tolerant phase the host immune response to infection is negligible. Therefore, the majority of infected children (86–90%) demonstrate minimal histologic changes on liver biopsy with evidence of mild inflammation only (Bortolotti et al., 1998). Although rare, children may develop serious sequelae during childhood,
including severe hepatitis, cirrhosis, and HCC (Chung et al., 1987). Individuals infected
during childhood who develop chronic hepatitis B are more likely to develop serious
sequelae in adulthood. Infection acquired in adulthood does not involve the prolonged
immunotolerance phase typical of early-life infection (Yuen et al., 2000).

1.3.1.2 Host Factors

Host gender affects disease progression; the ratio of males to females with
cirrhosis resulting from chronic HBV infection is approximately 2:1, and the incidence
of HCC is three to six times higher in men than women (Fattovich et al., 2003). Older
age is another risk factor for HCC, though it may be a reflection of prolonged duration of
HBV infection. The peak incidence for clinically evident cirrhosis or HCC is 50–60 yr of
age (Fattovich et al., 2003). The immune status of the patient also influences disease
progression, with more rapid progression in immunosuppressed patients than in those
who are immune competent (Lee et al., 1997).

1.3.1.3 Viral Factors

Viral load is a significant factor in the natural history of hepatitis B (Chin et al,
2003), with patients with active replication being at greater risk of disease progression
than those without detectable HBV DNA in serum. Cirrhosis results from prolonged
immune destruction of antigen presenting hepatocytes, and prolonged viremia may
influence disease progression. Risk of HCC is related in part to a direct effect of viral
replication and genomic integration, and in part to the host immune response including
necroinflammation and hepatic regeneration. A population-based prospective cohort
study of 3,774 Taiwanese patients with CHB investigated the relationship between serum
HBV DNA level and the risk for cirrhosis (Chen et al., 2005). A second study in Taiwan
addressed the association between persistent elevation of HBV DNA and the risk for
HCC (Chen et al., 2005).
1.3.1.4 Exogenous Factors

Coinfection with hepatotropic viruses (including hepatitis A, C, or D viruses) or HIV may influence the risk of disease progression in patients with chronic hepatitis B infection (Sagnelli et al., 2000). Immunosuppression after transplantation (liver, kidney, or heart) is associated with reactivation of disease and progression if HBV is not controlled (Gane et al., 2002). Similarly, reactivation of HBV as a result of cancer chemotherapy is a well-recognized phenomenon (Yeo et al., 2000). Heavy alcohol consumption can increase the risk of progression to cirrhosis and HCC (Hassan et al., 2002).

1.4 Clinical spectrum

1.4.1 Chronic Infection

Chronic HBV infection is defined as the presence of HBsAg in serum for at least six months or the presence of HBsAg and the absence of anti-HBc immunoglobulin M (IgM). Persons with CHB infection are at substantially increased risk of developing chronic liver diseases, including cirrhosis of the liver and primary hepatocellular carcinoma (McMahon et al., 1990). Persons with chronic HBV infection are generally classified as having one of three histologic patterns on liver biopsy: chronic persistent hepatitis, chronic active hepatitis, and cirrhosis. The degree of histologic injury is often not reflected by the symptoms and persons with severe chronic liver disease are often asymptomatic until late in the course of their illness (Hoofnagle et al., 1982).

1.4.2 HBeAg positive Chronic Hepatitis

Patients with HBeAg-positive chronic hepatitis B usually present in the third or fourth decade of life. Men outnumber women (Cacciola et al., 2002). Liver damage ranges from mild (24 to 42%) to moderate or severe chronic hepatitis (44 to 63%) or active cirrhosis (10 to 24%) (Di Marco et al., 1999). A key event in the natural history of
HBeAg positive chronic hepatitis is HBeAg seroconversion. Several studies have shown that seroconversion with marked reduction of HBV replication is associated with biochemical and histologic remission of inflammatory activity in the majority of patients (Fattovich et al., 1986). Regression of fibrosis occurs gradually months to years after HBeAg seroconversion (Fong et al., 1993). In longitudinal studies the observed probability of clearing HBeAg was about 50% and 70% within 5 and 10 years of diagnosis, respectively (McMahon et al., 2001).

1.4.3 HBeAg-negative chronic hepatitis

The diagnosis of HBeAg-negative CHB is based on the presence of HBsAg for more than 6 months, undetectable HBeAg, presence of anti-HBe, detectable serum HBV DNA exceeding $10^5$ to $10^6$ copies/mL, increased ALT levels and hepatic necroinflammation on histology. The atypical serological profile is related to the predominance of HBV variants, which are unable to express HBeAg. The most frequent variant has a G to A change at nucleotide 1896 (G1896A), which creates a stop codon in the precore region of the HBV genome and completely abolishes the production of HBeAg (Hadziyannis et al., 2001). Other variants include changes in the start codon of the precore region or a two-nucleotide substitution (A1762T, G1764A) in the core promoter region, which reduces precore messenger RNA synthesis and HBeAg production (Chan et al., 2000).

1.4.4 Inactive HBsAg Carrier State

The inactive HBsAg carrier state is diagnosed by absence of HBeAg and presence of anti-HBe, undetectable or low levels of HBV DNA, repeatedly normal ALT levels, and minimal or no necroinflammation, slight fibrosis, or even normal histology on biopsy (Lok et al., 2001). Long term follow-up (up to 18 years) of these carriers has indicated that the vast majority show sustained biochemical remission and very low risk
of cirrhosis or hepatocellular carcinoma (HCC) (Bellentani et al., 2002). Approximately 20 to 30% of persons in the inactive HBsAg carrier state may undergo spontaneous reactivation of hepatitis B during follow-up (Perillo et al., 2001; Hsu et al., 2002).

1.5 Long-term sequelae of chronic hepatitis B

1.5.1 Cirrhosis

Following the diagnosis of chronic hepatitis B, the survivals in these patients are estimated to be 100% at 5 years. In patients without cirrhosis, if untreated, the incidence of liver related death is low and ranges from 0 to 1.06 per 100 person years. In untreated individuals with predominantly HBeAg positive chronic hepatitis B, the incidence of cirrhosis ranges from 2 to 5.4 per 100 person years with a 5 year cumulative incidence of cirrhosis of 8% to 20% (Fattovich et al., 2003).

A higher rate of cirrhosis has been reported in HBeAg-negative as compared to HBeAg-positive patients. Also, older age and persistent viral replication are predictors for development of cirrhosis as well as mortality. The presence of any other independent hepatotoxic factors such as alcohol ingestion, HCV co-infection can contribute to progression to cirrhosis. Once cirrhosis is established, individuals can decompensate over time (Fattovich et al., 2002).

1.5.2 Hepatocellular carcinoma

The development of hepatocellular carcinoma and liver failure are main causes of death from chronic hepatitis B. HCC is one of the most malignant cancers, increasing by estimated 560,000 new cases per year, and the third among most common cause of death among men (Parkin et al., 2005).

HCC incidence is three to six times higher in males than in females (McMahon et al., 1990). The main causes of HCC are chronic infection with HBV, long term dietary exposure to aflatoxin B1 (AFB1), chronic alcoholism, besides other causes (Hussain et
Several studies have indicated that older age (>45 years) is an important determinant of HCC, this may either reflect a longer duration of viral infection and liver disease or age may be an independent risk factor. Having a first degree relative with HCC, the presence of cirrhosis and reversion activity is thought to contribute to HCC development (Benvegnue et al., 1994). Chronically infected subjects have a 100 times increased risk of HCC compared with non-carriers (Hsu et al., 2002). A study suggested positive HBsAg increased one’s risk of developing HCC by 10 folds, and with positive HBeAg, HCC is significantly increased by 60 folds. Moreover, a detectable HBV DNA level yields a 4 fold increase risk of HCC (Yang et al., 2002). Worldwide, the highest incidences of HCC and the youngest patients with this tumour are found in China, Taiwan and sub-Saharan Africa, each of which is hyperendemic for HBV infection with either HBV genotypes B, C (in China, Taiwan) or genotype A (in sub Saharan Africa) and a high rate of dietary exposure to the fungal toxin, AFB1 (Schaefer et al., 2005; Hussain et al., 2007). From a large number of molecular epidemiological studies, persuasive evidence has now accumulated that in HCC endemic regions, AFB1 and HBV interact synergistically in the aetiology and pathogenesis of HBV related HCC (Hussain et al., 2007; Stern et al., 2001).

Several large, long term, prospective cohort studies have linked high viral load with poor outcomes:

1. HaimenCityCohort - The study was a 10 year prospective cohort in Haimen city. There was a permanent cohort of 83794 subjects established between 1992 and 1993. 2,354 subjects were included in HBV mortality analysis. Serum HBV DNA was tested on baseline samples. 448 out of 2,354 deaths included (231 HCC, 85 CLD and 132 non-liver deaths). The Haimen City Cohort showed that high HBV DNA increases the relative risk of mortality from HCC and CLD (Chen et al., 2005).
2. Fox Chase Centre Cohort Study - 3754 infected Asian American adults in Philadelphia were recruited in a nested case control study. Patients with a viral load \( \geq 10^5 \) had a relative risk of 9.8 compared to 2.1 in patients with viral load \(<10^5\) (Evans et al., 2004).

3. R.E.V.E.A.L study - 89293 individuals in Taiwan were recruited in a prospective multicentre observational cohort study. It was found that HBV viral load was an independent risk factor for HCC. High viral load was associated with increased incidence of HCC. HBV viral load was an independent risk factor for cirrhosis (Chen et al., 2006).

1.6 Indian Scenario

Epidemiology of HBV infections in India

Infectious diseases are a major cause of deaths in South Asia, including India which now has the second largest population with AIDS and HIV infection in the world (Zaidi et al., 2004), signifying the rapid change in the epidemiology of parenterally/sexually transmitted viral infections via different modes (Gupta et al., 2006; Bhattacharya et al., 2007). According to the WHO report on prevention of HBV in India (WHO, 2002), HBsAg prevalence among general population ranges from 0.1% to 11.7%, being between 2% to 8% in most studies.

In contrast to the mainland India, very high rates of HBsAg have been recorded among certain primitive tribes of Andaman and Nicobar Islands. Studies showed hyperendemic HBV infection, with HBsAg carrier rates ranging from 23.3% among the Nicobarese tribe, 37.8% among the Shompen tribe (Murhekar, 2000), 11.6% among the Karen (Murhekar, 2004), and over 65% among the Jarawa tribe (Murhekar, 2003). The HBsAg prevalence rates among the Jarawa are the highest ever reported in the world.
### 1.6.1 Prevalence studies of hepatitis B markers in India

#### Table 1. Prevalence of HBV markers in age under 5 (Tandon et al., 1991)

<table>
<thead>
<tr>
<th>Age (Yr)</th>
<th>HBsAg+ve / total</th>
<th>%</th>
<th>Anti-HBs+ve / total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>8/320</td>
<td>2.5</td>
<td>42/249</td>
<td>16.9</td>
</tr>
<tr>
<td>1-3</td>
<td>8/353</td>
<td>2.3</td>
<td>44/309</td>
<td>14.2</td>
</tr>
<tr>
<td>4-5</td>
<td>5/309</td>
<td>1.6</td>
<td>123/838</td>
<td>14.7</td>
</tr>
</tbody>
</table>

#### Table 2. Prevalence of HBV markers in different age groups. Delhi (Panda et al., 1995)

<table>
<thead>
<tr>
<th>Age (Yr)</th>
<th>HBsAg+ve / total</th>
<th>%</th>
<th>Anti-HBs+ve / total</th>
<th>%</th>
<th>Anti-HBc+ve / total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>18/148</td>
<td>12.2</td>
<td>31/113</td>
<td>27.4</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>6-15</td>
<td>59/591</td>
<td>10</td>
<td>59/316</td>
<td>18.7</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>16-25</td>
<td>33/1303</td>
<td>2.5</td>
<td>94/480</td>
<td>19.5</td>
<td>200/659</td>
<td>30.3</td>
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<tr>
<td>26-35</td>
<td>260/7036</td>
<td>3.7</td>
<td>96/363</td>
<td>26.4</td>
<td>65/363</td>
<td>17.9</td>
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<tr>
<td>36-45</td>
<td>57/1160</td>
<td>4.9</td>
<td>28/95</td>
<td>29.4</td>
<td>15/95</td>
<td>15.8</td>
</tr>
<tr>
<td>&gt;45</td>
<td>3/50</td>
<td>6</td>
<td>5/34</td>
<td>14.7</td>
<td>3/34</td>
<td>8.8</td>
</tr>
</tbody>
</table>

ND: Not Detected.

#### Table 3. Data from the blood donors and pregnant women

<table>
<thead>
<tr>
<th>Place</th>
<th>Test used</th>
<th>Subjects</th>
<th>No.</th>
<th>% HBsAg+ve (95% CI)</th>
<th>Reference</th>
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<tbody>
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<tr>
<td>Delhi</td>
<td>ELISA</td>
<td>VBD</td>
<td>20435</td>
<td>2.6(2.4-2.8)</td>
<td>Irshad et al., 1994</td>
</tr>
<tr>
<td>Lucknow</td>
<td>ELISA</td>
<td>VBD</td>
<td>313</td>
<td>2.2(0.5-3.8)</td>
<td>Choudhary et al., 1995</td>
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<tr>
<td>Bombay</td>
<td>RPHA/ELISA</td>
<td>VBD</td>
<td>10433</td>
<td>2.02(1.7-2.3)</td>
<td>Elavia et al., 1991</td>
</tr>
<tr>
<td>Madras</td>
<td>MELISA</td>
<td>VBD</td>
<td>530</td>
<td>7.0(4.8-9.2)</td>
<td>Sumathy et al., 1995</td>
</tr>
<tr>
<td>Vellore</td>
<td>ELISA</td>
<td>VBD</td>
<td>582</td>
<td>0.7(0.02-1.39)</td>
<td>Singhvi et al., 1990</td>
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<tr>
<td></td>
<td></td>
<td>BD</td>
<td>7987</td>
<td>3.8(3.4-4.2)</td>
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<tr>
<td>Trivandrum</td>
<td>ELISA</td>
<td>VBD</td>
<td>143462</td>
<td>2.54 (1.1-3.6)</td>
<td>Shenoy et al., 2004</td>
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<tr>
<td>Pregnant Women</td>
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</tr>
<tr>
<td>Delhi</td>
<td>MELISA</td>
<td>-</td>
<td>8431</td>
<td>2.26(1.9-2.6)</td>
<td>Panda et al., 1991</td>
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<tr>
<td>Delhi</td>
<td>ELISA</td>
<td>-</td>
<td>8575</td>
<td>3.7(3.3-4.1)</td>
<td>Nayak et al., 1984</td>
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<tr>
<td>Chandigarh</td>
<td>ELISA</td>
<td>-</td>
<td>1000</td>
<td>2.3(1.4-3.2)</td>
<td>Kulkarni et al., 1988</td>
</tr>
<tr>
<td>Category / Place</td>
<td>Authors</td>
<td>HBsAg + Tested</td>
<td>HBeAg + No. (%)</td>
<td>HBeAg - No. (%)</td>
<td>Method</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------</td>
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<td></td>
</tr>
<tr>
<td>Chandigarh</td>
<td>Biswas et al (1989)</td>
<td>23</td>
<td>11(47.8)</td>
<td>12(52.2)</td>
<td>ELISA</td>
</tr>
<tr>
<td>Delhi</td>
<td>Panda et al (1991)</td>
<td>191</td>
<td>24(12.5)</td>
<td>167(87.5)</td>
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</tr>
<tr>
<td>Delhi</td>
<td>Mittal et al (1996)</td>
<td>54</td>
<td>10(18.0)</td>
<td>44(82.0)</td>
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</tr>
<tr>
<td>Mumbai</td>
<td>Gill et al (1995)</td>
<td>100</td>
<td>12(12.0)</td>
<td>88(88.0)</td>
<td>ELISA</td>
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<td>Thiruvananthapuram</td>
<td>Shanmugham et al (1983)</td>
<td>32</td>
<td>13(40.6)</td>
<td>19(59.4)</td>
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Table 5. Prevalence of CHB infection status in high-risk groups (HRG) in India.

<table>
<thead>
<tr>
<th>HRG/Place</th>
<th>Authors</th>
<th>Number tested</th>
<th>HBsAg positivity No./ Percent</th>
<th>Method</th>
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<tbody>
<tr>
<td>Hospital Personnel</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Chandigarh</td>
<td>Kant et al., (1995)</td>
<td>202</td>
<td>4.0</td>
<td>ELISA</td>
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<tr>
<td>Delhi</td>
<td>Irshad et al., (1994)</td>
<td>1313</td>
<td>1.4</td>
<td>CIE</td>
</tr>
<tr>
<td>Mumbai</td>
<td>Elavia &amp; Banker (1992)</td>
<td>863</td>
<td>10.0</td>
<td>ELISA</td>
</tr>
<tr>
<td>Mumbai</td>
<td>Kant et al., (1995)</td>
<td>114</td>
<td>7.9</td>
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<td>Thiruvananthapuram</td>
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<td>60</td>
<td>6.6</td>
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<tr>
<td>Chennai</td>
<td>Thyagarajan et al., (1981)</td>
<td>127</td>
<td>16.5</td>
<td>RPHA</td>
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<td>Chennai</td>
<td>Mohan et al., (1997)</td>
<td>78</td>
<td>64</td>
<td>ELISA</td>
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</table>

Family contacts

<table>
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<tr>
<th>HRG/Place</th>
<th>Authors</th>
<th>Number tested</th>
<th>HBsAg positivity No./ Percent</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pune</td>
<td>Arankalle et al., (1990)</td>
<td>-</td>
<td>39.0</td>
<td>ELISA</td>
</tr>
<tr>
<td>Chennai</td>
<td>Thyagarajan et al., (1996)</td>
<td>140</td>
<td>24.2</td>
<td>ELISA</td>
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STD Patients

<table>
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<tr>
<th>HRG/Place</th>
<th>Authors</th>
<th>Number tested</th>
<th>HBsAg positivity No./ Percent</th>
<th>Method</th>
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<tbody>
<tr>
<td>Chennai</td>
<td>Thyagarajan et al., (1992)</td>
<td>135</td>
<td>10.37</td>
<td>ELISA</td>
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</table>
2. **Hepatitis B virus**

2.1 **History**

The first recognition of a form of hepatitis that was transmissible through blood or blood products was reported by Lurman in Germany in 1885. In the late 1960s, a unique antigen was identified in the serum of an Australian aborigine patient with acute leukemia, and subsequently this antigen was found to occur most commonly in patients who had received multiple blood transfusions (Blumberg et al., 1965; Dane et al., 1970). The antigen was called the Australia antigen. When serum from Australia antigen-positive patients was studied by electron microscopy, vast numbers of spheres and filaments of 22 nm in diameter were seen, but also larger particles of 42 nm with a central nucleocapsid and an outer coat (Dane et al., 1970). The "Dane particles," were shown to be the etiologic agent responsible for HBV infection.

2.2 **Classification and Genomic Organization**

Hepatitis B virus is the prototype member of the Hepadnaviridae family. This family has been divided into two groups, the orthohepadnaviruses and the avian hepadnaviruses. These are hepatotropic, partially double-stranded DNA viruses. HBV is a remarkably compact virus with four open reading frames (ORF) (S, P, C and X) that encodes four major proteins (surface, polymerase, core, and X protein, respectively) (Figure 2). This compactness is achieved by the use of overlapping ORFs, so that more than one half of the nucleotides is used in a different frame for the transcription of different viral messenger RNAs (mRNAs) (Ganem & Varmus, 1987). HBV virions are double-shelled particles, 40 to 42 nm in diameter and are readily visualized by electron microscopy. HBsAg or S protein, which is 24 kD in size, is the major envelope protein of the virus. Two other proteins, L and M, which are 39 kD and 31 kD, respectively, are also present in the viral envelope. Within the envelope is a 27-nm structure known as the
nucleocapsid core (Robinson et al., 1976), which consists of 180 copies of the viral core protein, or hepatitis B core antigen (HBcAg), surrounding the viral DNA and the viral encoded polymerase. The core contains the viral genome, a relaxed-circular, partially duplex DNA of 3.2 kb and a polymerase that is responsible for the synthesis of viral DNA in infected cells (Summers et al., 1975).

The 5' ends of the DNA strands are each linked covalently to additional structures that are essential for the initiation of DNA synthesis: the polymerase, which is bound to the 5' end of the minus strand; and an oligo RNA, which is linked to the 5' end of the plus strand. Two short direct repeat sequences known as DR1 and DR2, which are present at the 5' ends of the plus and minus strands, are important for the initiation of DNA synthesis. The viral polymerase functions as both a reverse transcriptase for synthesis of the negative DNA strand from genomic RNA and an endogenous DNA polymerase (Summers et al., 1975). Two related proteins are produced by translation of mRNAs encoded by the core gene. One of these, the core peptide, is a 21-kD protein that forms the nucleocapsid core of the virus; the translation of the second protein, the hepatitis B e antigen (HBeAg), is initiated at a start codon upstream from the core AUG codon and consists of the core peptide plus a 30-amino-acid residue encoded by the precore region. The precore region contains a signal sequence that directs the protein to the endoplasmic reticulum, where host proteases cleave much of the C-terminus of the protein to form HBeAg. The function of the X protein has not been fully elucidated, but the X protein appears to be essential for replication because viral genomes with mutations in the X ORF fail to produce active infection in culture. X protein appears to function as a transcriptional activator that influences the transcription of HBV genes as well as those of other viruses (e.g HIV) by regulating the activity of transcriptional promoters (Rossner et al., 1992).
2.3 Viral Replication Cycle

The viral life cycle of HBV is relatively well characterized considering the lack of a robust and readily available permissive infection cell model, and can be considered in three stages: early, middle, and late (Locarnini et al., 2005).

2.3.1 The early stage

The early events in viral replication are poorly understood, and the cellular receptors (De Meyer et al., 1997) on the hepatocyte surface are still unknown until 2009.

2.3.2 Attachment, penetration, and uncoating

The first stage of infection involves attachment to a susceptible hepatocyte and the penetration of HBV into the cell cytoplasm following the binding of the HBV envelope to its specific cellular receptor/coreceptor. It has been assumed by most investigators that a process of receptor-mediated endocytosis, rather than membrane fusion, is responsible for the delivery of viral nucleocapsids into the cytoplasm.
Cytoplasmic viral nucleocapsids are then transported to the nuclear membrane, where they uncoat (Rabe et al., 2003). The genomic relaxed circular (RC) DNA is released into the nucleus and then converted into covalently closed circular DNA (cccDNA) using host cell enzymes, resulting in the formation of the viral minichromosome, the major template of HBV that is used for the transcription of all the viral mRNAs involved in viral protein production and replication (Newbold et al., 1995).

2.3.3 The middle stage

Five major RNA species, two of 3.5 kb, and one each of 2.4, 2.1, and 0.7 kb, are transcribed from the viral minichromosome. The transcripts can be classified into two classes: subgenomic and genomic (Ganem et al., 2001). The 2.4 and 2.1 kb mRNAs translate the large (Pre-S1), middle (Pre-S2), and small (S) envelope proteins. Both the Pre-S2 and S envelope proteins are translated from the 2.1 kb mRNAs. The Pre-S1 is translated from the 2.4 kb transcript and is required for the formation of the virions as well as the filaments. The smaller 0.7kb mRNA translates the X protein. The larger genomic transcripts, which measure 3.5 kb, are greater than one genome in length and serve as the pgRNA and precore RNAs. Reverse transcription is initiated upon binding of the viral rt polymerase to the encapsidation signal on the pgRNA. This is followed by the binding of core proteins to form nucleocapsids. A series of interactions results in the synthesis of minus-strand DNA-strand, which is then followed by positive-strand synthesis and circularization of the genome (Ganem et al., 2001).

2.3.4 The late stage

The assembly of nucleocapsids containing mature RC DNA occurs in the cytosol, and these nucleocapsids are selectively enveloped before exiting the cell. Completion of the (+) DNA strand typically occurs after re-entry of the virion into a host cell (Ganem et al., 2001). Enveloped nucleocapsids are deposited into the ER lumen and then move
through the Golgi, and the viral particles are secreted from the cell via the constitutive secretory pathway (Gerelsaikhan et al., 1996).

2.4 Genome Heterogeneity.

Hepadnaviruses have an unusual mechanism of viral DNA replication involving reverse transcription of pregenomic RNA by the virus-encoded polymerase (Nassal et al., 1996). Phylogenetic analysis of HBV full-length genomes has led to the classification of HBV into eight genotypes, A–H, and four major serotypes, ayw, ayr, adw and adr. The separate genotypes are arbitrarily defined by an intergroup divergence in the complete HBV genome sequence of more than 8% (Orito et al., 1989) and at the level of the S gene of more than 4% (Norder et al., 1992).

2.4.1 Subtypes

The first report of variability in HBV came from Le Bouvier (1971) who described two mutually exclusive subtype determinants, ‘d’ and ‘y’. These reside in the surface protein together with the main antigenic determinant ‘a’ (Levene & Blumberg, 1969). Two additional determinants, ‘w’ and ‘r’, were described by Bancroft et al. (1972), who found that each HBV strain could be characterized as belonging to either subtype adw, adr, ayw or ayr. An additional nine subtypes were described; ayw1 to ayw4, ayr, adw2, adw4, adrq+ and adrq− (Courouce-Pauty et al. 1983).

During the 1980s, it became increasingly clear that the subtype determinants are specified by one single amino acid, at positions 122 (d or y) and 160 (r or w) in the S protein, respectively (Ashton-Rickardt & Murray, 1989). Subtype determinants d and w have a lysine at both positions, whereas an arginine at both positions indicates subtype determinants y and r. Additional subtype determinant re-activities have been mapped to amino acid positions 127, 144, 145, 158, 159, 177 and 178 (Norder et al., 1992).
2.4.2 Distribution of HBV genotypes

The eight genotypes show a distinctive geographical distribution. Genotype A is prevalent in north-western Europe, North America and Africa (Norder et al., 1993; Westland et al., 2003). Genotypes B and C are characteristic of Asia (Okamoto et al., 1988), whereas genotype D has a worldwide distribution but predominates in the Mediterranean area (Lindh et al., 1997; Westland et al., 2003). Genotype E is found in Africans (Norder et al., 1994; Lindh et al., 1997; Odemuyiwa et al., 2001), genotype F in the aboriginal populations of South America (Norder et al., 1993; Arauz-Ruiz et al., 1997) and genotype H is confined to the Amerindian populations of Central America (Arauz-Ruiz et al., 2002; Sanchez et al., 2002). To date, the isolation of genotype G has been limited to HBV carriers in France and Georgia, USA (Stuyver et al., 2000), UK (Westland et al., 2003), Italy (Westland et al., 2003) and Germany (Vieth et al., 2002).

Figure 2 - Worldwide distribution patterns of HBV genotypes and subgenotypes.
Regions with high, intermediate and low endemicity are shown by grey, light grey and white shades respectively.
3. **Hepatitis B virus Genotypes**

Sequence heterogeneity is a feature of the hepatitis B virus (HBV) because the viral polymerase lacks proof reading activity (Akuta et al., 2003). The nucleotide substitution rate, per site per year, for HBV has been estimated to be $1.4 \times 10^{-5}$ to $5 \times 10^{-5}$ (Fares et al., 2002), approximately the same as retroviruses ($10^{-5}$) but $10^4$ times higher than DNA genomes (Orito et al., 1989). Viral mutations are controlled by functional constraints (Mizokami et al., 1997) and consequently variation is not random within the HBV genome (Yang et al., 1995).

3.1 **Individual genotypes**

3.1.1 **Genotype A and its subgenotypes**

Genotype A has a 6- nucleotide insert at the carboxyl terminus of the core gene that is not found in the other genotypes. Subgenotype A1 was identified in HBV isolates from South Africa using phylogenetic analysis of pre S2/S sequences (Bowyer et al., 1997) and confirmed by analysis of complete genomes from South Africa (Kramvis et al., 2002) and Malawi (Sugauchi et al., 2003). It has distinctive sequence characteristics in all ORF's and in the transcriptional regulatory elements (Kimbi et al., 2004). This subgenotype has also been found in Somalia (Sánchez et al., 2002), Brazil (Moraes et al., 1996), the Phillipines (Sugauchi et al., 2004), India and Nepal (Sugauchi et al., 2004) and Yemen (Sallam et al., 2004). With the exception of the Malawian (Sugauchi et al., 2003) and a few subgenotypes, A1 isolates from other countries were adw2. An aberrant genotype A, or possibly a separate subgenotype of A, had been identified in Vietnam (Hannoun et al., 2000).

3.1.2 **Genotype B and its subgenotypes**

Sugauchi et al., (2002) identified two subgenotypes of genotypes B. One subgenotype B1, the authentic genotype B is indigenous in Japan, whereas the other, B2,
predominating in Asia, had a genotype C recombinant region overlapping the precore / core ORF of the HBV genome (Sugauchi et al., 2003).

3.1.3 Genotype C and its subgenotypes

Using phylogenetic analysis of the pre S/S and the complete genome of limited number of HBV isolates, Huy et al., (2004) had classified genotype C into two subgenotypes, C1 and C2. Subgenotype C1 is found predominantly in Vietnam, Myanmar, Thailand and subgenotype C2 is found in Japan, Korea and China (Huy et al., 2004). Genotype C with C^{1858} (Alestitig et al., 2001), instead of the usual T^{1858}, has been shown to exist in 10-25% of genotype C carriers in East Asia (Lindh et al., 1997) and was initially proposed to be a separate phylogenetic entity that diverged several hundred years ago (Alestitig et al., 2001). However, subsequent studies have not supported this proposal (Sugauchi et al., 2002). Another variant or subgenotype of genotype C has been isolated from Australian Aborigines and is closely related to Polynesian strains (Sugauchi et al., 2001). The complete genome of the variant differed from that of the other genotype C strains by 5.9-7.4 %, whereas the small S gene differed by 4.0-5.6 % and the serological subtype was ayw3 which is not characteristic of genotype C (Sugauchi et al., 2001).

3.1.4 Genotype D

Genotype D is characterized by a 33 nucleotide deletion at the N terminus of the pre S1 region.

3.1.5 Genotype E

Genotype E does not separate from genotype D in the X and C ORF's (Bowyer et al., 2000) and its existence has been questioned (Kidd-Ljunggren et al., 1995). However, in the pre S region, genotype E isolates have a 3 nucleotide deletion at the amino terminus and genotype E signature amino acids, Arg^{39}, His^{45}, Thr^{53}, Met^{84}, Lys^{86} and
Thr$^{109}$ (Norder et al., 1994). The serotype ayw4, defined by Arg$^{122}$, Lys$^{160}$ and Leu$^{127}$ residues, is characteristic of genotype E. The nucleotide motif CCAGCTTCC (amino acids Pro Arg), 18 bp upstream from the core gene stop codon, is shared by HBV genotypes E-H and non-human primate hepadnaviruses. In the core region, genotype E has the Golgi peptidase motif AsnThrTrp↓Arg upstream from the arginine region instead of ThrThrTrp↓Arg found in all other HBV genotypes (Takahashi et al., 2000). The low genetic diversity of genotype E isolates over large geographic area, may be the result of a recent introduction of genotype E viruses into the human population (Mulders et al., 2004). Moreover its isolation from chimpanzees has led to speculation that genotype E may have once been a chimpanzee hepadnavirus (Takahashi et al., 2000).

3.1.6 Genotype F and its subgenotypes.

Genotype F diverges from the other genotypes by 14% and may represent the first split from the human hepadnaviral ancestor, the deduced amino acid sequences reflect the high genetic divergence of this genotype (Norder et al., 1994). In earlier studies, genotype F isolates from different geographical regions were shown to separate out into four different subgenotypes, previously designated as clusters I-IV (Mbayed et al., 2001), and two clades within cluster I after phylogenetic analysis of HBsAg genes and complete genomes (Devesa et al., 2004), although this was not found to be the case in a small number of genotype F isolates from Venezuela (Quintero et al., 2002). It became evident that genotype F strains in Central America have T$^{1858}$ and Thr$^{45}$ in the S gene, whereas those derived from South America and Polynesia have C$^{1858}$ and Leu$^{45}$ (Norder et al., 2003). These variants fall within two subgenotypes of genotype F, named: clade I (T$^{1858}$ Thr$^{45}$) and clade II (C$^{1858}$ Leu$^{45}$) by Norder et al., (2003). Clade I subgenotype F1 corresponds to cluster I (the nomenclature used by Mbayed et al. 2001) and clade 2 subgenotype F2 is composed of clusters II and IV (Mbayed et al., 2001).
Cluster III (Mbayed et al., 2001) corresponds to the newly described genotype H (Arauz-Ruiz et al., 2002).

3.1.7 Genotype G

Although variants with sequences characteristic of genotype G were identified as early as 1990 (Tran et al., 1991) and their abnormal viral protein expression demonstrated (Kato et al., 2002), the sequences were recognized to belong to a separate genotype only much later (Stuyver et al., 2000). The complete genome of genotype G is the least divergent from genotype E (11%) and the most divergent from genotype F (15%) (Kato et al., 2002). In contrast to other genotypes, the core region of genotype G has a 36bp insert, 3' of position 1905. In addition, like the other genotypes, it lacks the 6 bp insert on the carboxyl end of the C ORF found in genotype A isolates. Like Genotype E, it has a 3bp deletion in the amino terminal of pre S1. The precore/core region has two translational stop codons at positions 2 and 28, and therefore would not be expected to express HBeAg (Kato et al., 2002). However HBeAg has been detected in sera of patients infected with this genotype (Vieth et al., 2002) and it has been suggested that the secretion of core protein, which shares epitopes with HBeAg may be responsible for the HBeAg positivity (Vieth et al., 2002). The co-infection of genotype G with a wild type virus belonging to another genotype may account for the expression of HBeAg (Kato et al., 2003).

3.1.8 Genotype H

Nicaraguan isolates, which were previously separated as a subgenotype of genotype F after analysis of HBsAg sequences and referred to as cluster III (Mbayed et al., 2001), were found to differ from genotype F by 7.5-9.6% after analysis of the complete genomes. These strains were therefore shown to belong to a different genotype and designated genotype H (Arauz-Ruiz et al., 2002). Ten Mexican HBV isolates also
cluster within this genotype (Sánchez et al., 2002). Because genotype H is most similar to genotype F, it was concluded that this genotype has most likely split off from genotype F within the New World (Arauz-Ruiz et al., 2002).

3.2 Relationship of Mutations to Genotypes

3.2.1 1896 Stop Codon Mutation

The precore-core region of the HBV genome codes for the precore-core fusion protein that is post-translationally modified to give rise to hepatitis B e antigen (HBeAg) (Jean-Jean et al., 1989). The occurrence of the 1896 mutation is restricted by the secondary structure of the encapsidation signal (e) (Lok et al., 1994), which is transcribed from the same region of the HBV genome coding for HBeAg. Destabilization of this structure by the disruption of the G-C base pair between positions 1858 and 1896 (that would result from a G-to-A mutation at 1896) would be detrimental to viral replication (Lok et al., 1994), as has been shown by transfection experiments (Li et al., 1993). Thus the development of the 1896 mutation depends on the presence or absence of C or T at position 1858 and shows geographic variation that is related to the distribution of the various genotypes (Li et al., 1993). Genotypes B, D and E have T\textsuperscript{1858}, whereas A and H have C\textsuperscript{1858}. Genotype C and F isolates can have either C\textsuperscript{1858} or T\textsuperscript{1858}; genotype F strains in Central America have a T\textsuperscript{1858} (Arauz-Ruiz et al., 1997) and Japanese genotype C strains have T\textsuperscript{1858} exclusively (Alestig et al., 2001), whereas C\textsuperscript{1858} is confined to carriers of genotype C in South-east Asia (Sugauchi et al., 2002). Some studies found no difference in the prevalence of 1896 mutants between genotypes B and C (Sumi et al., 2003). It is of interest that although both subgenotypes of genotype B, identified in Asia (Ba and Bj) have T\textsuperscript{1858}, the 1896 mutation was found to occur more often in subgenotype Bj (Sugauchi et al., 2003). The 1896 mutation is found most frequently in anti-HBe positive patients infected with genotype D (Bozdayi et al., 2001).
and E (Suzuki et al., 2003) and is rarely found in genotype A (Jardi et al., 2004), genotype H (Arauz-Ruiz, 2002) and in a minority only of genotype C (Lindh et al., 2000) and genotype F strains (Norder et al., 2003).

3.2.2 \( T^{1762} A^{1764} \) Basal Core-Promoter Mutations

An adenine (A) to thymine (T) transversion at position 1762 together with a guanine (G) to A transition at 1764 in the Basal core promoter (BCP) were first described in HBV isolates from Japanese patients (Sato et al., 1995). The presence of the mutations precedes seroconversion from HBeAg to anti-HBe in genotype A strains but not in genotype D (Blackberg et al., 2000). Their presence results in reduced levels of precore mRNA and HBeAg expression in transfection studies (Gunther et al., 1998). The \( T^{1762} A^{1764} \) mutations develop more frequently in genotypes A and H with \( C^{1858} \), but in a minority of genotype C with \( C^{1858} \) and more often in subgenotype Ba than in Bj or genotype C (Sugauchi et al., 2003). However, in other analyses, they are equally distributed among the HBV genotypes (Jardi et al., 2004). Yet other studies show a higher frequency of the \( T^{1762} A^{1764} \) in genotype C compared with genotype B (Sumi et al., 2003) and does not correlate with HBeAg status (Sumi et al., 2003). Only 25% of carriers infected with genotype E possess the \( T^{1762} A^{1764} \) mutations and this finding was independent of HBeAg status (Suzuki et al., 2003). These mutations were found to be significantly associated with more severe liver disease, liver cirrhosis with or without hepatocellular carcinoma (HCC) and an older age (>35 years) (Orito et al., 2001).

3.2.3 Pre-S Mutants

Various mutations in the pre-S region have been described. These range from point mutations and small deletions and insertions to very large deletions (Gerner et al., 1998) and deletions that prevent the expression of the pre-S2 protein (Fernholz et al., 1993). A few studies have analyzed the relationship between genotypes and the
occurrence of pre-S mutants. In two independent studies, pre-S deletion mutants were found to occur more frequently in genotype C than in genotype B isolates (Sugauchi et al., 2003) and in adr (corresponding to genotype C) than in adw (corresponding to genotype B) isolates (Tai et al., 2002). In contrast, in another study, although these mutants are found more frequently in genotypes B and C than in the other genotypes, no statistical difference was found between their incidence in genotypes B and C (Huy et al., 2003). Deletion mutants are more frequently detected in isolates from patients with severe liver disease than other patients (Sugauchi et al., 2003). Deletions within the pre-S region can lead to impaired viral clearance without affecting HBV binding to hepatocytes and their subsequent penetration, and therefore could contribute to chronicity of infection (Ehata et al., 1991).

3.3 Recombinations between Different Genotypes

Mixed infection with different HBV genotypes is of great virological and clinical interests. Recombination events are a prerequisite for the reports of HBV isolates of two genotypes. However, concurrent infection with more than one genotype of HBV has infrequently been detected by genotyping methods which are unsuitable for the simultaneous detection of plural genotypes, probably due to one's presence being masked by a predominate strain. Using PCR-RFLP, the prevalence of mixed HBV genotype infections in Taiwan, an area highly endemic for HBV, was only 1.1% (Kao et al., 2002). Osiowy and Giles, (2003) detected mixed genotype infections in 10% of HBV carriers by the line probe genotyping assay. Based on suitable methods, such as genotype specific primers and line probe genotyping assays, it was found a high prevalence of mixed genotype infections (17.5% and 16.3%, respectively) in at-risk intravenous drug users (Chen et al., 2004). Recombination between 2 genotypes has been described in Asia where genotypes B and C are both endemic and in Africa where genotypes A and D are
both common (Chen et al., 2004). The recombinant HBV (HBV/Ba) is a chimera between genotypes B and C in which the majority of the genetic framework is HBV genotype B, except for the basal core promoter (BCP)/precore/core region which corresponds to genotype C (Sugauchi et al., 2002). This recombinant HBV occurs throughout Asia except in Japan. A different recombinant virus between genotypes B and C was described by Alestig et al., (2001). Recombinants between genotypes A and B in the S gene was found in an intravenous drug user who was coinfected with genotypes A and B (Chen et al., 2004). Full genomic sequencing and analysis of the recombination crossover points is the only method which can define and characterize newly recognized recombinant genotypes.

3.4 Genotypes and disease progression

The majority of studies on the effect of genotypes on disease progression have been undertaken in South East Asia where HBV is hyperendemic and genotypes B and C prevail. A greater frequency and severity of liver dysfunction was initially reported in patients infected with type ayr (mainly genotype C) compared with adw (mainly genotype D) (Noguchi et al., 1994). Seroconversion from HBeAg positive to anti HBe positivity occurs much earlier in genotype B than genotype C carriers (Ding et al., 2002). Higher HBV DNA levels have been detected in patients infected with genotype B in some studies (Ishikawa et al., 2002), but not in others (Sumi et al., 2003). Genotype C was found to have lower HBV DNA levels than genotype A, B and D in the HBeAg positive phase (Westland et al., 2003). In south western Japan carriers of genotype D were younger and exhibited earlier anti HBe seroconversion than carriers with genotype C (Duong et al., 2004). Patients infected with genotype B are more likely to have a sustained biochemical remission after spontaneous HBeAg seroconversion than patients infected with genotype C (Chu et al., 2002), who are more likely to develop chronic and
advanced liver disease (Sugauchi et al., 2002). Genotype C is more prevalent in patients with fibrosis or cirrhosis (Sumi et al., 2003) and is associated with more severe histological liver damage than genotype B (Chan et al., 2002) or genotype D (Duong et al., 2004). Patients infected with genotype C have higher scores of histological activity and fibrosis (Lindh et al., 2000), and higher alanine aminotransferase (ALT) levels relative to those infected with genotype B (Sugauchi et al., 2002), genotype A or D (Vivekanandan et al., 2004). The majority of studies in Far Eastern countries have shown a greater risk of HCC development with genotype C than with genotype B (Sugauchi et al., 2003). Patients infected with genotype B exhibit earlier HBe seroconversion and progress to liver fibrosis and HCC at a slower rate than those infected with genotype C, and it has been suggested that the life long risk of progression to advanced fibrosis and development of HCC may not differ among genotype B and genotype C related chronic liver disease (Yuen et al., 2003).

In contrast to genotype B found in Taiwan (Kao et al., 2000) and China (Ding et al., 2001), which is associated with the development of HCC at a young age, in Japan, the mean age of HCC patients infected with genotype B is significantly older than those infected with genotype C (Sumi et al., 2003). Although it has been suggested that this discrepancy between Chinese and Japanese HCC patients, could be a result of host factors and the intake of aflatoxin in Taiwan (Orito et al., 2001a), the difference is probably the consequence of the different subgenotypes found in mainland Asia (Ba) and Japan (Bj) (Sugauchi et al., 2002). When matched HBV carriers were compared, HBeAg positivity occurred in a significantly lower proportion of those infected with subgenotype Bj compared with Ba or genotype C and loss of HBeAg occurs earlier in carriers of Bj (Sugauchi et al., 2004). Subgenotype Ba occurred more frequently in acute than in chronic hepatitis patients (Sugauchi et al., 2004). Genotype A and D were found to be
prevalent in the Indian subcontinent. In one study genotype D was associated with more severe liver disease and with HCC in young patients (Thakur et al., 2002), whereas in another study, where the majority of patients were infected with genotype D, it was concluded that genotype D did not influence the clinical outcome of infection (Gandhe et al., 2003). Chronic infection with genotype A is more frequent than when individuals are infected with genotype D (Mayerat et al., 1999). Genotype A was more prevalent in HBeAg positive chronic hepatitis patients, whereas genotype D was more prevalent in those positive for anti HBe (Westland et al., 2003). HBeAg positive and HBeAg negative carriers infected with genotype D were found to have higher levels of HBV DNA when compared with genotype A, B and C (Westland et al., 2003). The prognosis of chronic Hepatitis B may be better in patients infected with genotype A than in those infected with either genotypes D or F because concomitant sustained biochemical remission and decrease in HBV DNA levels occurred at a higher rate in genotype A than in genotype D or genotype F infected patients (Sanchez et al., 2002). Genotype D was also found to be associated with severe recurrent disease post transplantation (McMillan et al., 1996). In a single study, genotype F infected individuals showed a higher mortality rate than those infected with genotype A or D (Sanchez et al., 2002). However this does not agree with other reports that showed a low pathogenicity of genotype F (Devesa et al., 2004). Very few isolates of genotype G have been characterized making it difficult to draw any conclusions regarding the influence of this genotype on disease progression. Chronic Hepatitis patients infected with genotype G are characterized by high HBV DNA and HBeAg levels (Westland et al., 2003) and elevated ALT levels (Kato et al., 2002).

4. Therapeutic Options for Chronic Hepatitis B

The goals of treatment in chronic hepatitis B virus (HBV) infection are sustained viral suppression, normalization of serum alanine aminotransferase (ALT) and
improvement in liver histology, leading to long-term reduction in the risk of cirrhosis and HCC. Currently, six drugs are licensed for treatment of chronic HBV infection: interferon alfa and pegylated interferon α-2a, which have antiviral and immunomodulatory activities and are administered subcutaneously (Hoofnagle et al., 2007) and pure antiviral agents; lamivudine, adefovir, entecavir, and telbivudine (Papatheodoridis et al., 2002). Pegylated interferon α-2b is licensed for chronic HBV infection in China, India, the Philippines, Turkey, Russia, and Brazil. All four antiviral agents are analogues of natural nucleosides (lamivudine, cytosine; entecavir, guanosine; telbivudine, L-deoxythymidine) or nucleotides (adefovir, adenosine) (Papatheodoridis et al., 2002). Treatment outcomes differ between patient groups, and the histologic and serologic characteristics of a patient with chronic HBV infection are not the only important considerations in the therapeutic decision-making process.

4.1 HBV Genotypes and Response to Antiviral Therapy

Correlations between HBV genotypes and response to treatment have been the focus of a number of recent studies. Patients with HBV genotypes C and D have a lower response rate to Interferon alpha (IFN-α) compared to those with genotypes A and B (Hou et al., 2001). A better response to IFN treatment occurred more often in genotype A than genotype D patients (Hou et al., 2001). The serological subtype response during lamivudine therapy was reported by Zollner et al. (2001), in which serotype adw was associated with a 20-fold increased risk of selection of lamivudine resistance compared with serotype ayw. A study showed that the risk of the emergence of lamivudine resistance-associated mutations was lightly higher during the first year in genotype A patients, but if therapy was prolonged to 2 or 3 years, the proportion of lamivudine-resistant mutations was the same, regardless of the HBV genotype (Buti et al., 2002). The HBV genotype does not seem to play a role in increased risk for lamivudine
resistance after 1 year of lamivudine therapy (Lok et al., 2000). Kao et al. indicated that genotype B seems to have a better virological response to lamivudine as compared to genotype C; however, both genotypes have a similar risk of developing drug-resistant mutants after 1 year of therapy (Kao et al., 2002).

5 Liver: The Organ

The liver is the largest solid organ in the body. The weight of a normal liver comprises about 1/18 of the newborn child’s body weight (approx. 5%) and about 1/50 of the adult’s body weight (2.3-3%), varying in men from 1,500-1,800g and in women from 1,300-1,500g (Ludwig et al., 1972). The liver is on average 25-30 cm in width, 12-20 cm in length and 6-10 cm in thickness. The surface is smooth and shiny. The color of the liver is brownish red. The normal liver occupies the right upper quadrant and extends from the fifth intercostal space in the mid-clavicular line to the right costal margin. The position is intraperitoneal. It lies below the diaphragm in the thoracic region of the abdomen.

5.1 Liver Topology

On a visual basis (topographically), the liver is divided into a large right lobe and smaller left lobe by the falciform ligament, which connects the liver to the diaphragm and the anterior abdominal wall. The right lobe is about six times the size of the left. Functionally, however, the liver is divided according to the distribution of its main vascular and biliary channels. The liver has a dual blood supply. The hepatic artery carries well-oxygenated arterial blood to the liver. The second source is the portal vein, which carries nutrient rich/ oxygen-poor venous blood to the liver from the intestines and spleen. About 50 to 80% of the liver’s oxygen supply is furnished by the hepatic artery; the remainder comes from the portal vein. Approximately 20% to 30% of hepatic blood flow is normally derived from the hepatic artery, with the portal vein contributing 70% to
80%. Nearly one-third of the cardiac output passes through the liver. The portal vein, hepatic artery and the common hepatic bile duct ascend to the hilum of the liver where each penetrates the liver parenchyma and bifurcates into right and left branches going to the anatomic right and left lobes, respectively. These vascular and biliary channels divide into progressively smaller branches, until they reach the “portal triads.” Each portal triad contains an artery, a portal vein, and one or two bile ducts.

6 Histopathology of the Liver

6.1 Microscopic anatomy

It is the “acinus,” that describes the functional unit of the liver. Each simple acinus actually encompasses segments of several lobules and agglomerates with adjacent acini to form more complex acini. As blood flows down the sinusoids, the progressive removal of oxygen and nutrients by hepatocytes depletes the supply available to hepatocytes further along the cords. Thus, within the acini, there are zones of successively less well oxygenated liver cells (zones 1, 2, & 3), which explains why systemic hypoxia preferentially damages the zone 3 hepatocytes near the central veins (Figure 4).

Hepatocytes are polygonally-shaped cells with extensive, microvillus-rich, basolateral surfaces, across which nutrients and oxygen are taken up from the space of Disse. Hepatocytes are richly supplied with mitochondria and rough and smooth endoplasmic reticulum, to support their extensive roles in energy production, protein synthesis and metabolism/detoxification respectively. Within the acinus, hepatocytes are normally arranged, like a string of boxcars, in cords or plates one or two cells thick, that are separated by the sinusoids. The space of Disse, which lies between the endothelial cell layer and the cords of hepatocytes, extends partially between the lateral surfaces of adjacent hepatocytes.
Figure 3- Anatomy of a Traditional Hepatic Lobule

Cords (plates) of hepatocytes, 1-2 cells thick, with intervening sinusoids are arranged radially around a central hepatic venule. At the periphery of the lobule are multiple portal triads, each containing a portal venule, a hepatic arteriole, and 1-2 intralobular bile ducts (Evaluation of Liver Function in Clinical Practice, Eli Lilly & Co., Indianapolis, IN 1965).

Figure 4- Anatomy of the Acinus, The Functional Unit of the Liver

The liver acinus consists of cords/plates of hepatocytes between which the sinusoids radiate from the terminal vascular stalk (terminal portal venule and hepatic arteriole). The hepatocytes nearest the terminal vascular stalk (zone 1) receive the highest concentrations of nutrients and oxygen, while the most peripheral hepatocytes in zone 3, near the terminal hepatic venules, receive the lowest concentrations of these substrates (Milner-Fenwick and Timonium, 1973).
6.2 Grading and staging

De Groote and Desmet (1968) introduced the first histological classification and codified the terminology chronic persistent and chronic aggressive hepatitis. In 1971, Popper and Shaffner affirmed the value of liver biopsy for diagnosis and prognosis. They recommended use of topographic descriptions for hepatitis, which were chronic lobular, chronic portal or chronic periportal hepatitis. Chronic, periportal hepatitis, synonymous with chronic aggressive hepatitis was believed to progress, whereas chronic portal hepatitis, synonymous with chronic persistent hepatitis, was considered as non progressive process. Desmet and Gerber (1994) replaced this classification taking into account the etiology, clinical and serological markers. The introduction of the scoring systems provided the foundation for the current understanding of the histopathology of chronic hepatitis. Ishak et al., (1976) and Bianchi De Groote et al., (1971) discussed the unique findings in chronic hepatitis B, including ground glass cells, liver cell dysplasia and hepatocellular carcinoma. In an attempt to standardize the interpretation of asymptomatic cases of chronic hepatitis and without specific consideration of etiology, Knodell et al (1981) described a semi quantitative method of scoring liver biopsies that grades four different sets of features, periportal necrosis (piecemeal necrosis) with or without bridging necrosis, parenchymal injury, portal inflammation and fibrosis. The summation of the individual component scores provides a total histology activity index (HAI). The first three features evaluate the necro inflammatory components of the disease, ie, the ‘grade’ and the fourth component fibrosis evaluates the histological stage which is the most important factor in prognosis.

Ishak’s 1994 review promotes the use of descriptive terminology for activity and fibrosis, rating the different elements of activity as either present or absent and when present, the degree of severity is stated. The modified HAI published by Ishak et al in
1995, introduced the term interface hepatitis instead of piecemeal necrosis to reflect the growing evidence that apoptosis and not necrosis occurs at the limiting plate (Hubscher et al., 1998).

The Knodell Histology Activity Index (HAI) is a semiquantitative and reproducible histological scoring system. Lesions are assigned numerical scores which are added up to get the final score. Ishak’s modified HAI grading is done under four different categories with a total score of 18. Staging by fibrosis allows a maximum of 6 scores. Relatively little information is available concerning the extent of agreement between different pathologists when reviewing the same biopsy specimen. Evaluation of liver biopsies is aimed at confirming the clinical and serological diagnosis, grading of necro inflammatory activity staging of consecutive fibrosis, ruling out or confirming liver diseases of different etiology and assessment of therapeutic effects.

7. **Steatohepatitis in Chronic Hepatitis B Infection**

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of conditions characterized histologically by excessive accumulation of hepatic fat in the absence of alcohol consumption. Two main histological patterns of NAFLD are described: fatty liver alone and Non Alcoholic Steatohepatitis (NASH). NAFLD is an increasingly recognized cause of liver-related morbidity and mortality (Angulo et al., 2002; Charlton et al., 2004). Although the majority of patients do not develop complications, 28% may develop serious liver sequelae, including end-stage liver disease and hepatocellular carcinoma. Those at highest risk include patients with significant hepatic necro-inflammation and fibrosis (Sanyal et al., 2002). Hepatosteatosis is defined as fat deposition in the liver that exceeds 5% of the total weight of liver, or with more than 5% of hepatocytes containing fat deposits under light microscopic examination (McCullough et al., 2004). On worldwide grounds, the prevalence of steatosis is very high, and is
associated with several factors such as alcohol, diabetes, overweight, hyperlipidemia, insulin resistance, hepatitis C genotype 3, abetalipoproteinemia and administration of some drugs (Levitsky et al., 2004). The impact of superimposed non-alcoholic fatty liver disease is well established in patients with chronic hepatitis C, but the impact in patients with chronic hepatitis B is less clear.

In addition to its own progression, superimposed NAFLD or NASH can affect the progression of other liver diseases. Hepatic steatosis may contribute to liver injury by increasing sensitivity to oxidative stress and cytokine-mediated hepatic damage. As noted, superimposed NAFLD and insulin resistance (IR) are associated with increased fibrogenesis in many studies on chronic hepatitis C (CH-C) patients (Lonardo et al., 2004). Preliminary data from HBV patients also suggest that hepatic steatosis in these patients may be related to host factors rather than viral factors (Thomopoulos et al., 2006).

8 HBV Genotyping Methods

A variety of methods have been used, including whole or partial genome sequencing, restriction fragment length polymorphism, genotype specific PCR amplification, PCR plus hybridization, and serology (Bartholomeusz et al., 2004). Whole-genome sequencing is the "gold standard," and it is particularly accurate for detecting recombinant viruses. However, it is cumbersome and time-consuming and has limited ability to detect mixed-genotype infections. The most common method of sequence-based HBV genotype determination has been searching of the GenBank database for homologous sequences using BlastN. This approach was noted to be complicated by the paucity of genotype-annotated HBV sequences within GenBank (Bartholomeusz et al., 2004); however, the deposition of a growing number of annotated sequences into the database has made this approach more practical. PCR plus
hybridization has been adapted into a commercial product (INNO-LiPA; Innogenetics). The amplification target lies in the major hydrophilic domain of HBsAg and is encoded within the pre-S1 gene, which has been found to be useful for genotype determination by direct sequencing. This method has several advantages over direct sequencing. It can effectively identify mixed infection, as 65% of mixed infections were verified by clonal analysis (Osiowy et al., 2003). Reasonable analytical and clinical sensitivity has been achieved with a modified protocol incorporating a number of different improvements to the recommended PCR plus hybridization procedure, including automated extraction (MagNAPure; Roche Diagnostics), single-round PCR, and uracil N-glycosylase (Qutub et al., 2006).

9. Counseling of the HBsAg Positive Patient

Patients who screen positive for HBsAg should be advised on lifestyle modifications to avoid additional insults to the liver and to minimize risk of transmission of the virus. The patient should receive education on the potential modes of transmission including sexual transmission, blood exposure, and vertical transmission (Lok et al., 2007). Close contacts should be tested for HBV and should be vaccinated if not immune (Mast et al., 2005). Additionally, patients with HBV infection should be tested for other relevant hepatotropic viruses including hepatitis A and hepatitis C viruses (Liaw et al., 2004). Hepatitis D virus should also be tested for, as coinfection with HBV is seen on occasions. HIV testing should also be done given that coinfection confers an increased risk of disease progression and may have an impact on the choice of antiviral therapy (Hoffmann et al., 2007). Abstinence from alcohol is recommended as there is no definitive amount of alcohol that is safe (Lok AS et al., 2007). Patients should be advised to avoid hepatotoxic medications if possible, with specific attention to herbal supplements and other over-the-counter agents.