Chapter 2

LITERATURE REVIEW
2. LITERATURE REVIEW

The Human Liver is one of the most important glandular organs in our body. It is the most important metabolic organ as it secretes bile necessary for the breakdown of food in the small intestine. It performs many functions important for cell metabolism. This incredible organ 'packages' nutrients into a usable form for cells in other parts of the body. It transforms toxic substances for excretion from the body. It may be the most abused organ in the body as modern life contributes toxins from drugs, smoke, alcohol, pollution and stress, which must be detoxified by the liver. There are three important diseases, which affect the liver; they are Hepatitis, Jaundice and Cirrhosis. Hepatitis means the inflammation of the liver. There are two types of hepatitis: Serum and Infectious.

2.1. History

Viral Hepatitis is a disease, which was first described, in the fifth century BC. Hippocrates in his description of epidemic jaundice was undoubtedly referring to persons with acute Hepatitis B virus infection as well as other agents, which infected the liver. Although outbreaks of jaundice have been described throughout history, many of the outbreaks have been due to Hepatitis A virus. The first documented evidence of a form of hepatitis that was transmitted by the direct
inoculation of blood or a blood product was by Lurman in Bremen, Germany, in 1883 during a small pox immunization campaign. Of the 1289, shipyard workers who had received the vaccine, which had been prepared from human lymph, 15% of them developed jaundice several weeks to 8 months later. (Lurman, 1855)

In the first part of the twentieth century, military personnel who had taken yellow fever vaccine during World war II had jaundice and it was linked to a specific lot of vaccine that contained human serum (Flaum et al., 1926; Neefe et al., 1946). A follow up study in the 1980's, revealed 97% of the recipients who had taken the vaccine containing human serum had serologic evidence of HBV infection while only 13% of persons who received the vaccine that did not contain human serum had serologic evidence of HBV infection thereby confirming that HBV was the cause for this outbreak (Seeff et al., 1987).

Blumberg et al., (1967) while searching for polymorphic serum proteins, discovered a previously unknown protein in the blood from an Australian aborigine. He termed this protein, Australia (Au) antigen. It became apparent that this antigen was related to type B hepatitis. By 1968, other investigators, notably (Prince, 1968; Okochi and Murakami, 1968), had established that the Au antigen (now known as the hepatitis B surface antigen or HBsAg) was specifically found in the serum of type B hepatitis infected patients.
Dane et al., (1970) found the virus-like particles in the serum of type B hepatitis infected patients. These particles were considered to be the hepatitis B virus (HBV). Other non-related hepatitis viruses were discovered later, but the hepatitis B virus retained its name. The viral nature of these particles was confirmed by detection of an endogenous DNA-dependent DNA polymerase within its core by Kaplan et al., (1973) and discovery of this polymerase allowed (Robinson and Greenman, 1974a; Robinson et al., 1974b) to detect and characterize the HBV genome.

2.2. Description of the agent:

Hepatitis B virus is a hepadna virus, a small DNA virus having a genome of about 3.2 Kb in length, which exhibits a partially double stranded circular configuration. There are four ORF’s within the HBV genome; preS/S, preC/C, P and X ORFs. The minus strand of the DNA is almost a complete circle and contains overlapping genes that encode both structural proteins (pre-S, surface and core) and replicative proteins (polymerase and X protein). The plus strand is shorter and variable in length.

The pre S/S encodes major S (i.e.) HBsAg alone, middle S [i.e. pre S2/HBsAg] and large S (pre S1/preS2/HBsAg). The middle S and large S polypeptides are carboxy-co-terminal extensions of the major S protein. The
major S peptide consists of p24/gp27 polypeptides, which make up about 80% of HBV envelope protein. The middle S contains gp33/gp36 polypeptides and represents about 15% of the envelope protein. The large S consists of p39/gp42 and constitutes only 5% of the envelope protein. The preC/C ORF encodes the viral nucleocapsid polypeptide [(i.e.) HBcAg)] and a related, secreted polypeptide referred to as Hepatitis B e antigen (HBeAg). Hepatitis B core antigen (HBcAg) is a p21 polypeptide that not only assembles to form the viral core, but also is involved in the packaging of viral nucleic acid during virus core assembly. HBcAg is formed by initiation of transcription at the second initiation site of the preC/C ORF. HBeAg is a p18 polypeptide produced from transcription initiated at the first initiation site of the preC/C ORF. This results in a peptide with a signal sequence for secretion that is further modified post-translationally by loss of the amino terminal signal sequences and part of the carboxy terminal sequences. The P ORF encodes the HBV DNA polymerase and encompasses approximately 67% of the genome, overlapping the three other ORF’s. The P ORF has three distinct domains; a terminal protein, RNase H and Reverse transcriptase. The X ORF encodes an X protein (pX) of 154 amino acids, which appear to function as a modulator of HBV transcription. In addition pX is a potent transactivating factor for several genes of the host including Class I and II human leukocyte antigen (HLA) and intra cellular adhesion molecule I.
2.3. Clinical consequences of Hepatitis B virus infection:

Acute Hepatitis

The clinical course of HBV runs similarly to that of Hepatitis A Virus (HAV) but tends to be more severe and may be associated with serum-sickness-like syndrome. The mildest attacks are asymptomatic and are detectable only by an increase in serum transaminase levels. Alternatively, the patient may be anicteric but suffer from gastrointestinal and influenza-like symptoms. These patients are likely to remain undiagnosed unless a clear history of exposure is available. The severity of infection may vary from the asymptomatic and icteric (from which recovery is typical) through to fulminant, fatal viral hepatitis. Icteric attacks in adults are marked by a prodromal period (typically 3 - 4 days up to 2 - 3 weeks) during which a patient feels sickly, suffering from digestive symptoms such as anorexia and nausea and may, in the later stages, have mild pyrexia. Other common symptoms are mild pyrexia, rigors, loss of desire to drink alcohol or smoke, malaise, and occasionally severe headaches. The prodromal period is followed by the darkening of urine and lightening of feces followed by the development of jaundice.

The consequences of acute HBV infection are highly variable. The incubation period range from 6 weeks to 6 months and the development of clinical manifestations is highly age dependent. New borns generally do not develop
clinical signs or symptoms, and infection produces typical illness in only 5 to 15% of children 1 to 5 years of age (McMohan et al., 1985). In older children and adults, only 33% to 50% are symptomatic. Fulminant hepatitis occurs in about 1 to 2% of persons with acute disease and has a case fatality ratio of 63 to 93%.

**Chronic HBV infection:**

Chronic HBV infection is defined as the presence of HBsAg in serum for six months or longer after initial detection (Sjogren, 1994). The risk of developing chronic infection varies inversely with age and is highest (up to 90%) for infants infected in the perinatal period (Hyams, 1995; McMohan et al., 1985). 25 to 50% of the children who are infected between 1 and 5 years of age develop chronic infection, compared to 6 to 10% of acutely infected older children and adults (Mahoney, 1999).

Adult acquired chronic HBV infection begins with a phase of immune clearance (Realdi et al., 1980; Hoofnagle et al., 1981). Symptoms may or may not be present and serum liver transaminases are usually elevated. HBV DNA is present in low concentrations in serum. Patients are HBeAg positive and liver histology reveals chronic hepatitis of varying activity often with lobular inflammation. HBcAg can be detected in the nuclei and cytoplasm, and HBsAg is commonly detected in the cytoplasm. During this phase, HBV replication declines, and spontaneous HBeAg to anti-HBe seroconversion can be observed at the annual
rates of 2.7% (Luis Viola et al., 1981) to 25% (Hoofnagle et al., 1981). Often a brief episode of significant elevation of serum ALT levels precedes seroconversion (Liaw et al., 1983). Transition from replicative to non-replicative infection may be smooth, rapid and clinically silent or prolonged, fluctuating and marked by recurrent exacerbations (Lok, 1992).

Once HBV infected cells are destroyed by the immune system, patients enter the third phase when the active replication ceases, and HBeAg protein disappears. Liver transaminases tend to normalize and patients have no or minimal symptoms. HBcAg can be detected in the liver tissue and typical ground glass appearance of the hepatocytes is observed. The HBV DNA gets integrated in the host's genome and the presence of HBV DNA in the serum is only detected by PCR. The intensity and the duration of the second phase will have determined the degree of long-term carriage. At this phase of non-replicative infection, the patient is seen as a “healthy” carrier (Hoofnagle et al., 1987) although residual liver damage may be established.

Chronic Hepatitis B virus infection is a leading cause of chronic liver disease worldwide. It is estimated that 15% to 25% of people with chronic Hepatitis B virus (HBV) infection will die prematurely from cirrhosis or hepatocellular carcinoma. Chronic HBV infection induces substantial direct and indirect costs, significant mortality and morbidity.
Extra Hepatic Manifestations

Extra hepatic manifestations of HBV are caused by deposition of complexes of viral antigen and antibody related to surface, core and e proteins of the virus. These conditions include polyarteritis nodosa, glomerulonephritic mucocutaneous vasculitis and mixed cryoglobulinemia (although this is more often related to hepatitis C) (Di Bisceglie, 1989; Shusterman, 1984). Beasley (1988) in Taiwan reported only one death due to chronic renal disease in his cohort of carriers. The clinical manifestations are recognized only in a minority of carriers, although persistence of HBV infection with glomerulonephritis has been reported to be high, in areas where HBV is endemic (Lai et al., 1987).

2.4. Pathogenesis:

The replication cycle of HBV begins with the attachment of the virion to the hepatocyte. The steps which are involved in viral entry, uncoating and nuclear translocation are unclear. Synthesis of the plus strand HBV DNA is completed inside the hepatocyte nucleus, and the viral genome is converted into a covalently closed circular DNA (cccDNA). The cccDNA is the template for transcription of pregenomic RNA and messenger RNA. The 3.5 Kb pregenomic RNA serves both as a template for reverse transcription of the first (-) strand HBV DNA as well as a messenger RNA for the production of nucleocapsid and polymerase proteins. Four
mRNA transcripts of known function have been identified as being involved in HBV transcription and translation. The longest (3.5Kb) is the template for genome replication and expression of precore/core and polymerase proteins. A 2.4 Kb transcript encodes pre-S1, pre-S2, and HBsAg, while a 2.1Kb encodes only pre-S2 and HBsAg. The smallest transcript (0.7Kb) encodes the X protein.

HBcAg and HBeAg are translated from a common gene. When transcribed, HBcAg is targeted to the endoplasmic reticulum, here it is cleaved, and HBeAg (the precore fragment) is secreted. HBcAg is essential for viral packaging and is an integral part of the nucleocapsid. It is not detectable in serum by conventional techniques; however, it can be detected in liver tissue in patients with acute or chronic HBV infection.

HBV replication begins with binding of the virus to the cell surface and penetration (Gerlich et al., 1993; Kann and Gerlich, 1997; Lau and Wright, 1993). The virus is transported without processing to the nucleus, where the relaxed circular DNA is converted to a covalently closed circular DNA (cccDNA), which in turn acts as a template for viral RNA synthesis. HBV DNA does not integrate into the host genome during the normal course of replication.

The cellular and humoral immune responses to HBV infection are complex. Most studies suggest that HBV is not directly cytopathic to infected hepatocytes
and that the cellular response to several viral proteins correlates with the severity of clinical disease and viral clearance (Chisari and Ferrari, 1995). Antibody response to the viral envelope antigens contributes to the clearance of the virus, the cytotoxic T cells mediate viral clearance by killing of the infected cells. It has been postulated that chronic HBV infection is related to a weak T-cell response to the viral antigens. Neonatal immune tolerance to viral antigens appears to play an important role in viral persistence among persons infected at birth, the basis of a poor T-cell response in adults is not well understood.

### 2.5. Diagnosis of Chronic HBV Infection.

Serologic testing is the cost effective and definitive means of diagnosis in chronic HBV infection as the clinical symptoms of HBV is difficult to distinguish from other forms of hepatitis. Acute HBV infection is detected by diagnosis of anti HBCIgM. HBsAg detection has evolved from latex agglutination tests, RPHA, ELISA and to the most sensitive assays like the radioimmunoassay which detects HBsAg even at very low levels of >0.1 ng/ml. HBeAg is also detected during acute infection. During convalescence, HBsAg and HBeAg are cleared, anti HBs, anti HBC and anti HBe develop. Anti HBs is a protective antibody and it neutralizes the virus. The presence of anti HBs following acute infection indicates recovery and immunity from reinfection. Anti HBs is also seen in persons who have received hepatitis B vaccine. Anti HBC (Total) indicates current or past
exposure and viral replication. Anti HBcIgG appears shortly after HBsAg among persons with acute HBV and persists throughout life. Therefore anti HBc (Total) is not a good marker for persons with acute infection, only anti HBc (IgM) must be tested. In persons with chronic HBV infection, HBsAg remains persistent throughout life and anti HBcIgM becomes undetectable after six months of acute infection.

HBV DNA detection is of limited diagnostic value. HBV DNA is detectable in both acute and chronic infection. (Hadziyannis et al., 1983; Hoofnagle and Di Bisceglie, 1991; Kaneko et al., 1990; Ulrich et al., 1989; Vyas et al., 1991). HBV DNA by PCR is more sensitive than slot or dot blot hybridization assays and detects HBV DNA levels of approximately 100 to 1000 genomes, however PCR are prone to false-positive results. Detection of HBV DNA by hybridization indicates significant viral replication and a high probability of active liver disease (similar to that of HBeAg). Monitoring of the levels of HBV DNA is useful in the determination of the response of treatment in chronic HBV infection.

2.6. Global epidemiology of Chronic Hepatitis B virus infection:

In much of the world, in particular sub-Saharan Africa, East Asia and the pacific Basin, infection with hepatitis B virus (HBV) is very widespread.
Everyone experiences HBV infection at some point in their lives and large portions (perhaps 10-20%) of the population are carriers of the virus.

In two NHANES surveys II and III it was observed that the overall age adjusted prevalence of hepatitis B virus infection was 5.5% (95%CI 4.8-6.2) in NHANES II, as compared to 4.9% (95%CI 4.3-5.6) in NHANES III (McQuillan et al., 1999). Prevalence of chronic Hepatitis B virus infection approaches 10% in hyperendemic areas such as South east Asia, China and Africa. In North America and Europe, it is seen less frequently and in the United States 1.25 million persons are infected (Malik and Lee, 2000). In the United States, approximately 4000 to 6000 new infections occur each year in children and adolescents and they become infected without obvious clinical manifestations (Ott and Aruda, 1999).

Knowledge of the epidemiology, prevention, pathogenesis, natural history and treatment of chronic hepatitis B virus infection were made in the last 30 years. Globally there are currently 300 million carriers worldwide who are risk of dying from liver failure or hepatocellular carcinoma, and there will continue to be new cases of HBV infection for many more years (Lok, 2000).

In Africa and Egypt, hepatitis B and C infection rates differ in different settings and prognosis may be worse in conjunction with schistosomiasis in Egypt, Malaria in Sudan, and HIV in other African populations (Attia, 1998). In 1984/85 prevalence of anti HBs/HBc in German health care workers (12.4%) was 2.5 times
higher than the one in non-health care workers (4.9%). In 1994/95 anti HBs/HBe prevalence in both the groups (4.4 Vs 4.5) were comparable. On the other hand, HBV carriage in persons occupied in professions without blood contact increased from 1984/85 (0.5%) to 1994 / 95 (1.1%). Therefore, the number of HBV carriers (ca.1.1 millions) in Germany can be roughly estimated (Kralj et al., 1998). In Italy, the incidence of hepatitis B virus is 10 per 100,000 population, with most cases occurring in young adults (Germinario et al., 2000). Asia and Africa have previously been classified as areas of high endemicity for HBV but now China is the only country in Asia where HBV endemicity is high. Countries with intermediate endemicity include India, Korea, the Philippines, Taiwan and Thailand and those with low endemicity include Japan, Pakistan, Bangladesh, Singapore, Sri Lanka and Malaysia. Most countries in Africa have high endemicity, with exceptions of Tunisia and Morocco, which have intermediate endemicity. The Middle East, Bahrain, Iran, Israel, Kuwait are all areas of low endemicity. Cyprus, Iraq and UAE have intermediate endemicity while Egypt, Jordan, Oman, Palestine, Yemen and Saudi Arabia have high endemicity (Andre, 2000).

In Latin America, the highest seroprevalence of antibody to HBV core antigen was found in the Dominican Republic (21.4%) followed by Brazil (7.9%), Venezuela (3.2%) and Argentina (2.1%). The lowest seroprevalence was found in Mexico (1.4%) and Chile (0.6%) (Tanaka, 2000). In Poland, effective vaccination programs have reduced the incidence of Hepatitis B from 45 cases per 100,000
population to fewer than 15 per 100,000 in 1993. In 1997, only 32 cases in total were reported. (Magdzik, 2000). Cadranal et al., (1999) reported that 1,50,000 individuals are infected with HBV in France and that 30% of them have chronic hepatitis.

2.7. Epidemiology of chronic HBV infection in India:

Epidemiology of hepatitis B in India, is greatly hampered by the non availability of a national level network of surveillance reporting system, and analysis by the study of scattered reports is the best possible solution, to arrive at an consensus prevalence rates in India. Many variables in the reports like (1) incomparable designs of studies (2) sample size differences (3) incomparable methods adopted by investigators from different parts of the country (4) compelling need for indexing HBV prevalence in each and every part of India to obtain the national scenario, necessitating the inclusion of even poorly designed studies over the years with smaller sample sizes.

A review of the literature on the HBsAg positivity rate suggests a prevalence range of 1.1 to 12.2%. Based on the HBsAg positivity rate, Jammu and Kashmir & Kerala came under the below 2% zone, Karnataka, Maharastra, Delhi, Haryana, Himachal Pradesh, West Bengal showed 2.4% HBsAg carrier prevalence. Tamil Nadu, Pondicherry, Andhra Pradesh, Madhya Pradesh, Uttar
Pradesh & Himachal Pradesh belonged to high prevalence zones of above 4%. However the mean HBsAg carrier rate for India from these reports is 3.34%, which numerically works out to be about 40 millions with the base line population being 1000 millions in India. The point to be remembered besides the formidable pool of HBV infected population in India is the significant regional variation within the country (Thyagarajan et al., 1996).

Community prevalence studies are considered as better denominators for assessing the disease burden in a country than screening the blood donor population, which represents the general population. Chowdhury et al., (1999) in one of the rare community based study in West Bengal on 960 study subjects revealed a 5.3% HBsAg positivity rate.

The replicative status of HBV in the general population as denoted by HBeAg positivity based on the seven reports revealed a range of 2.6 to 56.1% with a mean rate of 24.22%. Similar to HBsAg prevalence, HBeAg prevalence also showed regional variation within India, with Maharashtra having below 10% replicative HBV status, Delhi between 10-20% rate while Tamil Nadu, Kerala and Andhra Pradesh coming under the above 20% zones. The replicative status of HBV in the chronically infected community population has been studied by Chowdhury et al., (1999) in West Bengal and the positivity was 3.9%. The risk of exposure to HBV infection in the high-risk groups in India ranges between 1.4-
45% in different regions approximately 5-20 times more common in the general population (Thyagarajan et al., 1996).

2.8. Familial clustering of Hepatitis B virus infection:

The first case of the detection of a family with Hepatitis-Associated antigen (HAA) was studied by (William Bancroft et al., 1971). Hepatitis associated antigen was detected in the serum of a five and half year old male infant with giant cell hepatitis. Three of the four other members of the family were symptomatic carriers of HAA with normal or nearly normal tests of liver function tests and no history of liver disease was observed. Reeves et al., (1975) observed that the infection rate in antigenic index families was 0.64 in contrast to 0.19 in infected families without antigen carriers. (P<0.001). Peters et al., (1976) in his study suggested that minute abrasions during sexual intercourse might be important in explaining the spread between spouses.

Scott Mazzur and Norman Jones (1977) are of the opinion that HBsAg carrier state in the family are apparently determined by cultural and environmental variables which increase the degree of exposure. Marcel Eliakim et al., (1978) demonstrated that HBsAg clustering occurred in families of patients with cirrhosis in the Jerusalem area and indicated that HBsAg negative family contacts may have increased B cell activity. A study by Perrillo et al., (1979) emphasized the
important role of spouse contact in the transmission of hepatitis B virus, presumably via sexual exposure or by intimate contact.

Alan Leitchner et al., (1981) observed that horizontal nonparenteral spread of Hepatitis B probably accounted for the clustering of infection in these families especially via the exchange among children of objects contaminated with oral secretions among children. Hess et al., (1979) in his study found that nearly one third of all the household contacts of asymptomatic HBsAg carriers had signs of past or ongoing infection and all family members run about equal risk of acquiring HBV infection. An study which demonstrated that HBeAg and DNA polymerase positivity increasing the prevalence of HBV infection in family contacts of HBV carriers was done by Pastore et al., (1981). Although the authors are of the opinion that infectivity markers increased the risk of HBV infection in family contacts of HBsAg carriers, such clustering may be due the individual immune response to HBV, or might be controlled by some unidentified polygenic inheritance.

In a large dynamic study by Juan Carlos Porres et al., (1989), a total of 848 household contacts of 285 hepatitis B surface antigen (HBsAg) chronic carriers were included. It was demonstrated that the prevalence of HBV marker was significantly higher among the contacts of more than one HBsAg carrier (75.9%) than among those with only one (26.0%) (P<0.001). Moreover, the presence of hepatitis B e Antigen, specific HBV-DNA polymerase, HBV DNA and
polymerised human serum albumin (pHSA-R) in the index case was associated with a significantly higher incidence of HBV markers among household contacts.

A study by Antonio Craxi et al., (1991) suggested six models of risk expressed as odds ratios, which were assessed by multivariate step-down analysis, with the following results.

1) Infection with HBV in the household contact was independently predicted by the index case being son, a sibling, spouse, female or HBV-DNA positive.

2) Chronic HBsAg carriage in the adult household contact was associated with female sex of the index case and with being a sibling, among young subjects, household contacts were more likely to be chronic HBsAg carriers when the index case was the mother, a sibling, or an HBV DNA positive subject.

3) HBV-DNA positivity in the young contact was more likely when the index case was HBV-DNA positive and when she was the mother.

4) HBV-DNA was not related to a similar pattern of infection in HBsAg positive contacts.

5) Superinfection with HDV of an HBsAg positive household contact was significantly predicted by female sex of the index case and by anti-HDV positivity.

6) Chronic liver disease in a contact was predicted only by HDV superinfection of the index case.
Judith Barrett (1976) studied the subtype prevalence of HBV in Australian aboriginal populations and found that subtype ay is prevalent. The family clustering was striking in all populations where the genealogies were known. Powell et al., (1985) reported about a family extensively affected by HBV in whom sexual or vertical transmission was unlikely to account entirely for the intra family clustering of the virus. HBV DNA was detected in the saliva of one family member and the authors suggest that spread by salivary transmission could account for at least some of the intra-family infection and could contribute to the “inapparent parenteral” spread of hepatitis B.

In a study in Taiwan, (Sung and Chen, 1978) found clustering of hepatitis B surface antigen with both subtypes adr and adw in three families with chronic liver diseases. In the family units where clustering was seen with different subtypes, invariably the fathers carried the HBsAg / adr and the children carrying the HBsAg / adw subtype. This subtype difference clearly rules out the transmission of HBV from father. Horizontal infection is most likely in these cases. Ohbayashi (1982) has shown that familial occurrences of liver cirrhosis and hepatocellular carcinoma are frequent in Japan. These occurrences according to the author occur mainly from mother to infant, and that many of the infected persons become HBV carriers.
Sanjay Dhorje et al., (1985), undertook a study to detect the risk of infection, if any, among 193 household contacts of 40 hospitalised patients (Group I) with HBsAg. 103 household contacts of 27 hospitalised hepatitis patients who were negative for HBsAg (Group II) were investigated. The family contacts of the Group I had a significantly high prevalence of HBV infection than those of the Group II. Overall, the children of the first group showed a significantly higher prevalence of HBsAg as compared with the second group. All the children with HBsAg were positive for HBeAg also, but were negative for anti-HBc IgM. A significant difference (P< .025) was noted in the number of families having HBV markers in Group I, (80.0%) as compared to those in Group II (48.1%).

Habu et al., (1991) analysed the relationship between the presence of intrafamilial clustering of infection with HBV and the condition of the liver. The subjects in the group without clustering had a higher rate of being seronegative for HBeAg than the groups with clustering.

Ramia (1990) analysed 10 Saudi families and concluded that both perinatal and horizontal modes of transmission is prevalent in the ten families and these results could be important for developing a strategy for controlling HBV in Saudi Arabia. In a epidemiological study by Ala Toukan et al., (1990) among family members in the middle east, history of contact with a jaundiced person and socio economic status were independent risk factors for HBsAg positive status, while
contact with a jaundiced person, rural background, and age were independently related to HBV infection. There was a trend towards an association of HBsAg positive children with HBsAg positive mothers. Post natal early childhood transmission through contact among children of poorer and larger families probably accounts for the high endemicity of HBV in this region. (Wang, 1993) in the Hebei province classified the family clustering of HBV infection on the basis of subtyping into six types namely, generational, horizontal, recessive, intra-and extra familial, nonfamilial and undetermined ones.

Yao (1996) suggests that in China, horizontal transmission is an important route of HBV infection during early childhood, and the proportion of chronic HBsAg carriage attributable to perinatal transmission has been estimated at only 13-20%.

Studies from Africa are very rare in the aspect of familial clustering of HBV. In a single isolated study by Abdool Karim et al., (1991) household contacts of 28 hepatitis B surface antigen (HBsAg) positive children, 22 hepatitis B surface antibody positive children (index-past-infection) and 35 children with no evidence of HBV infection (Index-Negative) were analysed. Evidence of HBV infection (at least one positive HBV marker) was present in 73.7%, 48.7% and 38.2% respectively in the three groups. Crowded living conditions and low socio
economic status may have provided an appropriate milieu for the spread of HBV infection.

2.9. Purification of Hepatitis B surface antigen:

Purification of Hepatitis B surface antigen has been carried out as early as 1972 (Dreesman et al., 1972). In order to prepare potent anti Australia antigen sera of high specificity and sensitivity in goats, three methods of purification were carried out. The first method included pelleting, low pH treatment, isopycnic centrifugation two times in CsCl and rate zonal centrifugation in sucrose. The second method was the same as the first procedure except that the low pH treatment was not carried out. The third procedure was done by twice banding in CsCl by using a BXIV batch type zonal centrifuge rotor with subsequent preparative Pevikon electrophoresis. Edda De Rizzo et al., (1972) purified hepatitis B antigen by a simple two step procedure. The antigen is precipitated by polyethylene glycol and then adsorbed on and eluted from an insoluble polyelectrolyte, polyelectrolyte 60, which is a cross-linked copolymer of isobutylene maleic anhydride. This procedure can be adapted in concentration and purification of smaller volumes so that it can be conducted in the clinical laboratory with a minimum of apparatus and time.
Chung Yong Kim and Jeremiah (1973) purified Hepatitis B antigen (HBsAg) from serum by pepsin digestion and equilibrium sedimentation. The sedimentation coefficient, density, particle size and electrophoretic mobility have been determined before and after purification and were found unaltered. They have also documented the diffusion constant and molecular weight of the purified HBsAg. The density of HBsAg was found to be 1.170 in sucrose, 1.216 in Cesium chloride, the particle size was found to be 22nm (range 14-38 nm), the diffusion co-efficient at 20°C was found to be 2.278x10^{-2} \text{cm}^2 \text{s}^{-1} and molecular weight was found to be 2.4x10^6. The problem of purification of HBs antigen arises from the relationship between this antigen and various serum proteins. The technique followed by Pillot et al., (1975) includes the following steps, adsorption of HBsAg on Silica (Aerosil 380) at 20°C, with successive washings until all proteins have completely disappeared from the washings; an elution performed at 37°C with Borate buffer 0.01 M, pH 9.3 of adsorbed HBsAg, gathering of the Sephadex G 200 excluded peak containing HBsAg: separation in sucrose gradient (10-50%) by zonal ultracentrifugation: a CsCl (1.26 g/ml) isopycnic ultracentrifugation. Even under these conditions, the above mentioned serum proteins are never completely eliminated.

Dreesman et al., (1975) purified HBsAg by centrifugation in CsCl twice and the second isopycnic density centrifugation was followed by a rate zonal
centrifugation for 5 hr at 36,000 rpm at 4°C. The purification of HBsAg was done with an aim to produce antibodies to specific polypeptides derived from HBsAg. A simple inexpensive procedure for the isolation and purification of HBsAg from plasma was carried out by Nath et al., (1976). The technique included precipitation of HBsAg with PEG and elimination of normal plasma proteins by digestion with Pepsin. Tween 80 was used to remove contaminating lipoproteins. This technique resulted in a 200-fold gain in the specific activity of HBsAg and yielded 20-40% recovery.

Johnson et al., (1976) used batch fractionation methods with polyethylene glycol to remove Hepatitis B surface antigen from plasma fractions. Wai-Kuo Shih and Gerin (1977) studied the proteins of Hepatitis B surface antigen for their constituent polypeptides by sodium dodecyl sulfate polyacrylamide gel electrophoresis. They purified the 22 nm form from serum components and other HBsAg forms by two cycles of isopycnic banding in cesium chloride followed by rate-zonal centrifugation in sucrose and a final banding in CsCl.

Monica Einarsson et al., (1978), purified HBsAg from plasma by affinity chromatography on matrix bound sulphated carbohydrates such as heparin or dextran sulphate. Further purification by precipitation with polyethylene glycol (PEG) 6000 was carried out. The overall recovery amounted to 70% of the total antigen content of the starting plasma. The process is simple, rapid and lends
itself readily to large-scale applications. Stamm et al., (1979) used the three procedures of gel chromatography, isopycnic centrifugation and zonal centrifugation to purify HBsAg from human plasma. The final product had about 60% of the original activity and was essentially free from hepatitis B virus particles (HBV) and plasma proteins. Treatment with formaldehyde concentrations up to 1% for inactivation of residual activity did not significantly reduce antigenicity in vitro and immunogenicity in guinea pigs.

Mc Auliffe et al., (1980) in a review on current prospects for a safe and effective vaccine against HBV, says that vaccines which are used are derived from plasma of chronic carriers and that they are inactivated with formalin. Moreover, extensive testing of these vaccines in humans and chimpanzees showed that these vaccines to be free from residual HBV or other harmful agents and to be capable of stimulating protective antibody production in a majority of recipients.

The most efficient method of purification of HBsAg is described by Mishiro et al., (1980). Briefly, HBsAg particles were purified by three cycles of rate zonal ultracentrifugation. First, plasma was made to a density of 1.30 g / cm$^3$ by adding an appropriate amount of KBr and subjected to floating type ultracentrifugation in a linear density gradient of KBr in a Hitachi RPZ 30T zonal rotor. Fractions with a density from 1.20 to 1.23 g / cm$^3$ were pooled, dialyzed against Tris-HCl buffer (0.01M, pH 7.5), concentrated by negative pressure
ultrafiltration, and subjected to a rate zonal centrifugation in a stepwise (10 to 50%) sucrose density gradient in the same rotor. Fractions enriched in respect to 22-nm spherical HBsAg particles were collected and subjected again to floating type ultracentrifugation in the KBr density gradient.

Fractions with a density from 1.21 to 1.22 g/cm³ that contained a high concentration of 22-nm HBsAg particles were collected, dialyzed against Tris-HCl buffer, concentrated and stored at -20° C until used. Monica Einarsson et al., (1981a) used hydrophobic interaction chromatography from a concentrate of coagulation factors II, VII, IX and X to remove deliberately added hepatitis B surface antigen. Chromatography in high concentrations of salt, preferably on octanoic acid hydrazide-Sepharose 4B, resulted in a $10^4 - 10^5$ fold reduction of HBsAg. Monica Einarsson et al., (1981b) purified hepatitis B surface antigen by a simple, rapid two-step procedure comprising chromatography on dextran sulphate-Sepharose 4B, followed by preparative zonal centrifugation on a sucrose gradient. A 230- fold purification of HBsAg was achieved with a yield of about 80%.

In order to characterize the major protein and glycoprotein of hepatitis B surface antigen, (Darrell Peterson, 1981) used the following procedure to purify HBsAg. The first step was Ammonium sulfate fractionation, followed by hydroxyapatite chromatography, then Ammonium sulfate precipitation. The following step was agarose 4B chromatography and then ultracentrifugation in two
32 ml Cesium chloride gradients and then followed by KBr floatation. An overall yield of 50% is obtained, based on counter electrophoresis titer, and approximately 3-5mg of HBsAg was obtained from 100 ml of high titer plasma. This purification procedure has the advantage of being applicable to large quantities of plasma without the need for the use of zonal rotors and large quantities of cesium chloride. The entire procedure is readily performed in four days.

Edward Tabor et al., (1983) inactivated dilutions of human sera containing $10^4$ and $10^5$ chimpanzee infectious doses of hepatitis B virus per ml, with 1g / ml pepsin at pH 2.0 for 18 hours, 8M urea for 4 hours, or 1:4,000 formalin for 72 hours. Moreover, one ml of the serum containing hepatitis B virus subjected to each of these procedures was injected into one or two susceptible chimpanzees. No evidence of infection was seen in any of the chimpanzees after six months of observation. These data substantiates the fact that these procedures used along with physical purification of the vaccine is capable of removing hepatitis B virus and that there is no residual live hepatitis B virus in the vaccine.

Mc Aleer et al., (1983) devised an artificial capillary system for the growth of hepatoma cells that yields high titers of hepatitis B surface antigen (HBsAg). High yield of the antigen was facilitated by slowing cellular metabolism through reduction of incubation temperature and addition of 0.1mM Caffeine. Moreover, the deletion of the serum from the medium did not reduce the yield of antigen.
HBsAg prepared from the culture fluid by affinity chromatography and additional chemical and enzymatic steps was essentially pure and was indistinguishable from HBsAg prepared from infected human plasma. Preparation of HBsAg from the cell culture source presents advantages over that of human plasma and might be a source of HBsAg for vaccine preparation. Thomssen et al., (1983) stressed the fact that residual infectivity in HBV vaccines can be prevented only by the use of plasma from healthy HBsAg carriers who are anti HBe positive. In a analysis done by Scheier (1984) during a follow up of about 20,000 vaccine recipients over a seven year period, no known occurrence of AIDS or other infectious disease have been associated with the vaccine. The plasma-derived vaccine for HBV is recommended to all people at high risk in Western Germany by the health organizations as well as by the WHO.

Adamowicz et al., (1984) in order to demonstrate the safety of hepatitis B vaccine, the efficiency of six steps used in the preparation process to remove or destroy pathogens was determined. Infectivity of all the tested viruses was reduced $10^5$ fold to a factor of $10^9$ fold by the first and the last steps namely PEG fractionation and formalin treatment. The four successive zonal ultracentrifugations decreased virus infectivity by atleast $10^7$ fold. Five of these steps also tend to purify the hepatitis B surface antigen, which increases the HBsAg: protein ratio by atleast $10^5$ fold.
In another method described by Govender et al., (1985) for the rapid isolation of purified HBsAg from infected donor plasma, polyethylene glycol precipitation and a single isopycnic ultracentrifugation step in which the gradient is formed in situ is employed. This technique works well using a small swing out rotor as well as a zonal rotor. This procedure is based on the principle that when two (or more) different concentrations of solute layered on top of each other (in a centrifuge tube or a zonal rotor) are allowed to diffuse, a gradient will be generated. The time required for the gradient to form is dependent on a number of parameters including the difference in the initial concentration of the two components. In a study done by Yoshida et al., (1986) to evaluate HBsAg positive plasma as a source for vaccine, the purification was carried out in the following manner. A volume of 1600 ml of plasma was passed through an affinity chromatography column with Sepharose 4B linked to goat anti-HBs antibody and partially purified antigen was obtained after elution with 4 M MgCl₂. Further purification was done by ultracentrifugation. Fractions of 0.8ml were collected and each was tested for protein content from the absorbance at 280 nm titrated by RPHA and the density by refractometry.

Howard Fields et al., (1988) evaluated five published methods used for purification and compared their specific activity, degree of purification and yield. HBsAg purified by the procedure of Mishiro et al., (1980) which included two consecutive isopycnic ultracentrifugation separations in KBr and one rate zonal
separation in sucrose using a zonal rotor yielded a preparation which gave the highest SA value, degree of purification and yield as compared to four other methods. The five methods evaluated included Dreesman et al., (1972); Nath et al., (1976); Darrell Peterson et al., (1981); Monica Einarsson et al., (1978) and Mishiro et al., (1980).

Kim et al., (1990) used an immunoaffinity method for the purification of HBsAg using monoclonal anti-idiotype (anti-Id) as a specific eluent. Ammonium sulfate fractions of HBsAg containing plasma were adsorbed on monoclonal idiotype (Id) immunoadsorbents. The bound material was eluted from the column with the anti-Id of HBsAg. The eluate containing anti-Id was further purified by gel filtration. The final HBsAg product was free from contaminating human plasma and mouse ascites proteins. The procedure is generally applicable and particularly attractive when the antigen is unstable under commonly used elution conditions.

2.10. Epidemiology of Chronic Hepatitis B virus infection and HCV in school children:

Chronic HBV infection acquired during childhood manifests itself as a chronic disease more efficiently than those who acquire the infection during adulthood. Reports have shown a steady increase in the prevalence rates in the older age group children between 16-19 years before declining again. A study from South Africa has shown that the HBsAg carrier rate increased after the first
year reaching 12% at age 5 and 54 % by age 15. There was a corresponding increase in the prevalence of anti HBs (Di Bisceglie et al., 1986). The same pattern of infectivity has been reported from Nigeria, and in the pacific Islands, where in addition to vertical transmission, horizontal spread from sibling, and among students at school may be the reason for the rise in infection in the older age group children (Fakunle et al., 1981; Chris Maher et al., 1991). Also important is the role of sexual transmission in the age groups of 16 to 19 years, where horizontal transmission also plays an equally important role in the transmission of chronic hepatitis B virus infection (Peter Skinhøj et al., 1979).

Interestingly a study from Philippines by Lingao et al., (1986) showed two patterns of age specific HBsAg prevalence rates “an early HBsAg peak” exhibiting a rapid rise among four year olds, followed by a gradual decline in the other age groups and a “late HBsAg peak” that had no HBsAg positive children until age four years, then showed a gradual rise which peaked at 30-39 years and declining thereafter. The reason for the latter pattern was related to the positivity rates of the mother at the time of birth.

Nayak et al., (1987) showed that majority of the HBsAg positive mothers are HBeAg negative. This study showed a 3.7 % maternal HBV carrier rate, 18.6 % infant infection rate during the first six months of life and 25 % loss of infection by the first year of age. This study also pointed out that large number of carriers
were getting chronic HBV infection outside the perinatally infected route and that there is no need for vaccination of neonates in contradiction to reports from Western countries.

The risk of evolution to chronicity in new borns is greater than 90% whereas only 5% or less of adults develop chronic infection. Moreover, a significant proportion of these infected new borns develops a poor prognosis. In addition, carrier status in childhood facilitates intrafamilial transmission, particularly to siblings, as well as transmission in pre-school day care centres (Esteban, 1995). The role of horizontal transmission in infection during childhood and adulthood has been well documented in Africa, Greenland, Spain, Italy and New Zealand. In all the places the main route of transmission has been sibling to sibling contact, intra-familial spread, school contact and horizontal nonparenteral spread (Williamson et al., 1985; Milne and Christopher Moyes, 1983).

Chiaramonte et al., (1991) in their study on healthy urban school children in Cameroon showed a HBV positivity of 35.9% for any HBV marker in the age group of 4-6 yrs. They showed that the prevalence of any HBV marker and HBsAg increased steadily with age suggesting that continued infection occurs even during school age, in contrast to observations made by a study in Italy, where transmission in the school age group was not significant (Chiaramonte et al., 1991; Chiaramonte et al., 1983). James Oleske et al., (1980) demonstrated the case of a
patient who acquired HBV infection from a classmate who was also HBsAg positive and remained unknown till this study. He also showed that both the children belonged to the same subtype.

Jun Huyashi et al., (1987) showed that none of the HBsAg positive children had parents who were positive for HBsAg or HBeAg. Infection seems to have been acquired from personnel who were working with the children all of whom were positive for the viral markers. Although the exact mechanisms of person to person transmission of hepatitis B virus is yet to be clarified, occurrence of non-parenteral transmission has been reported in class room setting and in institutions for mentally retarded children (Charles Williams et al., 1983). Whittle et al., (1990) in their study on HBV infection in two Gambian villages showed that HBV infection was infrequent in the first year of life. Sibling to sibling transmission rather than vertical transmission was thought to be of major importance. One interesting fact was that in one of the villages, a greater percentage of children infected at an older age became chronic carriers than those infected at a younger age.

2.11. Epidemiology of HCV in Children.

Globally, the incidence of HCV infection is low, compared to the adult population. Studies by Ndumbe and Skalsky, (1993) and Ngatchu et al., (1992) in
Cameroon have observed no positivity in urban children less than four years of age, but 14.5% positivity in children in the age groups between 4 to 14 years. In another study by Martinson et al., (1996) in school children of Ghana, a positivity of 5.4% was observed out of 803 samples tested for Anti HCV.

Studies in China by Fang et al., (1993) in the Wuhan province revealed a 2.9% in a day care home while the prevalence was very low 0.13% in Taiwan (Chang et al., 1993). In Spain the anti HCV positivity was 0.36% (Gil Miguel et al., 1996). In a study from Pakistan by Agboatwalla et al., (1994) the anti HCV positivity was 0.44% out of 258 samples tested. Even in studies from Egypt by Darwish et al., (1996) where HCV infections are widespread, they usually occur in adulthood.

Studies in India on the prevalence of Anti HCV in India are rare. Arankalle et al., (1995) found that only one out of 830 subjects was found positive for anti HCV antibodies revealing a low seroprevalence of 0.12% in Western India. Further the study showed that none out of the 86 children below five years of age tested positive for HCV which further reflects that vertical transmission is very rare from this area. The other report available from India is that of Singh et al., (1991) where none of the 206 healthy children tested for anti HCV were positive.
2.12. Traditional medicine systems in the treatment of Chronic Hepatitis B virus infection:

Review of research data on the traditional medicinal systems, which are being used for Chronic Hepatitis B virus reveals four plants that are being used. They are *Silybum marianum, Picrorrhiza kurroa, Glycyrrhiza glabra, Phyllanthus amarus* (niruri).

*Silybum marianum*: Silymarin has been shown to have clinical applications in the treatment of toxic hepatitis, fatty liver, cirrhosis, ischaemic injury, radiation toxicity and viral hepatitis via its anti-oxidative, anti-lipid peroxidative, antifibrotic, anti-inflammatory, immunomodulating and liver regenerating effects (Luper, 1988).

*Glycyrrhiza glabra*: *Glycyrrhiza glabra*. Linn belongs to the family Fabaceae and is cultivated in Punjab and in the sub Himalayan tracts. The plant is a tall perennial under shrub about 1 m high, leaves compound, leaflets 4-7 pairs, flowers violet in racemes, pools, oblong to linear, flattened, seeds reniform. The liquorice of commerce is the dried underground stems and roots. Its outer surface is pale chocolate brown in colour, flexible and fibrous and internally has a light yellow colour. It has a characteristic pleasant sweet taste.
The roots of the plant are used and the roots are sweet, refrigerant, emetic, toxic, diuretic, mild laxative, aphrodisiac, trichogenous, expectorant, emmenagogue, alexipharmic, alterant and intellect promoting. They are also useful in hyperdipsia, cough, bronchitis, urelcosis, vitiated conditions of vata, gastralgia, cephalalgia, fever, skin diseases, ophthalmopathy and pharyngodynia. The extract of the root is good for treating gastric ulcers. A decoction of the root is a good wash for falling and greying of hair, and externally the root is applied for cuts and wounds.

Glycyrrhizin has been identified as the active principle of *Glycyrrhiza glabra* and significant research has gone into this molecule internationally. Glycyrrhizin particularly has been found to have induction as well as hepatocyte protective effect. (Abe *et al.*, 1982; Shiki *et al.*, 1992; Nagai *et al.*, 1992). Glycyrrhizin is also reported to have antiviral properties through endogenous interferon induction as well as hepatocyto protective effect. Endogenous interferon induction by Glycyrrhizin has been documented both in mice (Abe *et al.*, 1982) and human (Shinada *et al.*, 1986). Its hepatocyto protective effect has been clearly documented in invitro hepatocyte culture system and ischemia reperfusion liver models in rats (Nagai *et al.*, 1992). Clinical trials in sub-acute hepatic failure and fulminant hepatic failure using Glycyrrhizin have shown significant patient survival. However, antiviral effects in chronic Hepatitis B were insignificant (Fujisawa *et al.*, 1973; Suzuki, 1983). Clinical trials using Glycyrrhizin among
patients with chronic hepatitis due to HCV have documented normalisation or decrease in ALT values as well as histological improvement (Fujisawa et al., 1973; Suzuki, 1983; Yasuda et al., 1991). Glycyrrhizin has also been shown to inhibit RNA viruses through a hitherto unknown mechanism (Pompei et al., 1979). Glycyrrhizin is a safe drug with minimal side effect. Therefore, it is rational to consider that combination of Interferon α2b with Glycyrrhizin which may have synergistic effect achieving better virological clearance and histological improvement among patients with chronic hepatitis C.

**Picrorrhiza kurroa:** *Picrorrhiza kurroa* belongs to the family: Scrophulariaceae is a perennial herb, found in the Alpine Himalayas from Kashmir to Sikkim at altitudes of 2700-4500 mts. The plant is used as a bitter toxic, anti periodic and cholagogue. Roots are stomachic, used in dyspepsia and fever; also used in snakebite and scorpion sting and in purgative preparation. The roots are also included in diseases of liver and spleen including jaundice and anemia.

Picroside 1 and 3, Catapol, Kutkoside I and Kutkoside have been identified as major bioactive components of *Picrorrhiza kurroa*, (Vaidya et al., 1996; Mehrotra et al., 1990), *Picrorrhiza kurroa* (PK), a known hepatoprotective plant, has been studied in experimental and clinical situations. Basic research has been carried out on the whole plant, Picroside 1 along with Picroliv, which contains Picroside 1, Catapol, Kutkoside I and Kutkoside.
Dwivedi et al., (1990, 1991a, 1991b), have shown significant hepatoprotective properties of Picroliv using models like monocrotaline induced hepatic damage in rats, thioacetamide induced hepatic damage in rats and also against CCl₄ induced liver damage in rats. Shukla et al., (1991) comparing Picroliv with Silymarin in rat and guinea pig models have shown potent choleretic and anticholestatic functions. Chander et al., (1990) evaluating the hepatoprotective activity of Picroliv in Mastomys natalensis infected with Plasmodium found that parameters of liver function and decrease in the levels of lipid peroxides and hydroperoxides and facilitated the recovery of superoxide dismutase and glycogen. However, Picroliv did not have any effect on the degree of parasitaemia.

Mehrotra et al., (1990) using invitro HBsAg binding assay have shown HBsAg inhibition in Picroliv and its major component, Catapol. Pandey and Das, (1988, 1989); and Atal et al., (1986) have shown anti-inflammatory and immunomodulating potentials of Picrorrhiza kurroa stimulating both cell-mediated and humoral immunity.

Jayaram in his Ph.D. thesis entitled “Studies on prevention and control of Hepatitis B virus infection” (University of Madras, 1992) had conducted a double-blind clinical trial on 4 groups of chronic carriers of HBsAg (carriers in all) who were treated under code with Phyllanthus amarus, Picrorrhiza kurroa, a combination of P. amarus and P. kurroa with the fourth group treated with lactose
Plate 1  :  Phyllanthus amarus
as placebo. The existing literature on *P. kurroa* confirms it more as a powerful immunomodulator than as an antiviral in liver diseases.

*Phyllanthus amarus*: *Phyllanthus niruri* Linn as it has been indexed in majority of published ethano botanical reviews, until recently, belongs to the family Euphorbiaceae. *Phyllanthus* is one of the largest genera of the family: Euphorbiaceae containing 700 species. It has been shown that about 24 species of *Phyllanthus* are active against clinical hepatitis (Jaundice) as indicated out of which 8 have been used in India.

The following species of Phyllanthus have been used in clinical jaundice. They are *Phyllanthus niruri, Phyllanthus amarus, Phyllanthus fraternus, Phyllanthus mimicus, P. debilis, P. urinaria, P. carolinensis, P. abnormis, P. airy-shavii, P. tenellus, P. gasstroemi* and *P. gunni*. This plant has recently been delineated as a mixture of three distinct species namely *Phyllanthus amarus, Phyllanthus fraternus* and *Phyllanthus debilis*. It was later identified that the circumtropical weed *P. amarus* is the predominant species in South India, particularly in Tamilnadu.

The first ever designed invitro antiviral study on *Phyllanthus niruri* against any hepatitis virus was done by Thyagarajan from Madras, India (1979). Subsequently Thyagarajan *et al.*, (1982) have shown that the whole plant extract
of *P. niruri* through several solvents brought about binding of Hepatitis B surface antigen (HBsAg). These plants from Tamil Nadu, India were taxonomically identified by Unander as *P. amarus*. Venkateswaran *et al.*, (1987) from the United States using the *P. amarus* plants provided by Thyagarajan have shown that the plants collected from Madras, India whose aqueous extracts bound the surface antigen of HBV invito, have inhibited the viral DNA polymerase (DNAp) of HBV and Woodchuck hepatitis virus (WHV) invito. When administered intraperitoneally to WHV infected woodchucks acutely infected animals lost the viral surface antigen and the surface antigen titre dropped in some chronically infected animals; the liver cancer rate in treated chronically infected animals was lower than the treated controls.

Yanagi *et al.*, (1989) from Japan have reported that the aqueous extracts of high dilutions of *P. amarus* collected from South India inhibited HBV DNAp, DNAp, T4-DNAp, the Klenow fragment and reverse transcriptase of avian mycoblastosis virus. Shead *et al.*, (1990) from Australia have shown the aqueous extracts to inhibit the endogenous DNAp of DHBV at high dilutions. Niu *et al.*, (1990) from Australia in collaboration with Thyagarajan from India using *P. amarus* collected from Madras, Tamil Nadu on treatment of 4-5 week old ducks congenitally infected with duck hepatitis B virus (DHBV) with suitable controls after a period of 10 weeks treatment showed transient reduction of viral DNA in serum but no effect on the level of virus DNA or surface antigen in the liver.
Jayaram et al., (1996) reported the invitro inhibition of HBsAg secretion by PLC/PRF/5 (Alexander) cell line for 48 hrs when the cell line was treated with 1mg/ml concentration of P. amarus as a single dose. Lee et al., (1996) from USA in collaboration with Thyagarajan have shown that P. amarus down-regulates Hepatitis B virus mRNA transcription and replication using transgenic mice and transgenic cell lines. The continuation of this collaboration by Ott et al., (1997) have shown that cellular and molecular mechanism of HBV suppression by P. amarus to be by interrupting interactions between HBV enhancer I and cellular transcription factors.

The biosafety studies on P. amarus dates back to 1971 when Mokkhasmit et al., (1971) from Thailand using P. niruri have reported it to be non toxic to mice at 10gms/kg body weight. Rao (1985) from Andhra Pradesh, India reported 20% aqueous extract of P. niruri leaves to be effective as oral pretreatment of 0.2 ml/100mg body weight against CCl₄ induced hepatotoxicity in rats. Syamasundar et al., (1985) from Uttarpradesh, India showed that the hexane extracted compounds Phyllanthin and Hypophyllanthin reduced CCl₄ or galactosamine induced cytotoxicity to cultured rat hepatocytes. Jayaram et al., (1987) from Madras, India using the aqueous extract of dried whole plant showed no chronic toxicity in mice at 0.2 mg daily dose per animal for 90 days as revealed by physiological, biochemical and histopathological parameters. There was also no
cytotonic changes when tested in a tissue culture model using vero cell line. Venkateshwaran et al., (1987) from USA demonstrated its invivo safety using woodchucks as animal models, while Niu et al., (1990), from Australia have shown that P. amarus to be non toxic in Pekin ducks chronically infected with duck Hepatitis B virus. Jayaram et al., (1994) while studying the effect of P. amarus on β- galactosamine induced hepatotoxicity on isolated rat hepatocytes have shown that a) P. amarus by itself did not bring about any hepatotoxicity on rat hepatocytes. b) At 1 mg/ml concentration the aqueous extract were shown to protect isolated rat hepatocytes significantly from β-galactosamine induced hepatotoxicity thus proving the anti hepatotoxic potentials of P. amarus.

In all the traditional medicinal systems, there have been formulatory medicines for the treatment of jaundice in general without taking into consideration their viral etiology. Even though P. niruri was one of the constituents of such medicines, these always have been multiherbal preparations containing anywhere upto 12 medicinal herbs and most of the treatment evaluations were based on clinical improvement only. On the other hand, there is no documented trial report on their use in chronic liver disease patients. It was in this context, Thyagarajan and collaborators, after proving the invitro and invivo efficacy and safety of P. amarus, conducted 2 open clinical trials in acute viral hepatitis cases and seven clinical trials (2 of them being double blind trials and others Phase I / II open trials) in chronic carriers of Hepatitis B virus. Jayanthi et al., (1988) in a control clinical trial in acute viral hepatitis (AVH) using P. niruri
on one arm, and other herbal medicines in other groups have shown a significantly
greater decrease in transaminases after two weeks treatment with P. niruri in both
HBsAg positive and negative groups. In a virologically characterised AVH
clinical trial, Geetha et al., (1992) have shown that a) P. amarus treatment has
brought about significantly faster biochemical normalcy in both hepatitis A and B;
b) there was a higher rate of HBsAg clearance in P. amarus treated AVH-B cases
than other treatment modalities and c) there was no observable side effects due to
P. amarus treatment.

The clinical trials were conducted by Thyagarajan et al., between 1988 and 1997. While the first trial of 1988 reported 59% HBsAg clearance in the P. amarus treated group, as against 4% in the placebo group, the second open trial of 1990 showed 20% HBsAg clearance and 63.6% loss of infectivity indicated by HBcAg sero-conversion. Parallely, investigators from other countries like Leelarasamee et al., (1990) from Thailand, Wang Me Xia, (1991) from China and Milne et al., (1994) from New Zealand have reported the non-reproducibility of treatment efficacy by the local variety of P. amarus grown in their respective countries.
2.13. Prevention:

Active Immunization:

HBV vaccines

The first commercially available vaccine, which was licensed by WHO in the early 1980s was plasma, derived HBsAg subunit vaccine (Szmuness et al., 1982). Although this vaccine provided excellent immunogenicity and protective efficacy, it was not widely accepted because of unnecessary concerns of other bloodborne infectious agents. The most common vaccine available in the market today is derived from a recombinant yeast source. The small hepatitis B surface protein (SHBs) is generated by the yeast cells. Expression of this protein by yeast results in SHBs particle formation. However, particles are not secreted by the yeast. Disruption of yeast cells is performed in order to liberate the produced spheres into solution. These particles are then purified through clarification, ultrafiltration, chromatography and ultracentrifugation. The purified particles are then adsorbed onto aluminium hydroxide to which thiomersal is added to preserve the solution. The two yeast derived vaccines licensed in most countries are Engerix-B (Smithkline Beecham, Philadelphia, PA) and Recombivax HB (Merck & Co., West Point, PA). Both products are structurally and chemically similar with less than 2% yeast protein remaining in solution. Recombivax HB, however, is treated with formaldehyde before its adsorption onto alum. As both are yeast-derived, the S-protein is not glycosylated (as yeast does not possess the correct
post-translational machinery to do so). Both, thankfully, appear to be quite effective as vaccines, allowing for immunization against the various forms of HBV.

The vaccines, however, should not be frozen, as this appears to be deleterious to its immunogenicity. Studies have shown that freezing of these vaccines results in lower immune responses. There are also other forms of immunization and vaccines. However, the ones mentioned above generally appear to be the most effective and the most widely used. Typical vaccination schedules are 0, 1 and 6 months or 0,1, 2 and 10 months. The 0,1, and 6 month vaccination schedule is preferred for routine pre-exposure prophylaxis. The four dose schedule may be preferred for immuno-compromised patients or for post exposure prophylaxis. It has also been recommended that a booster dose be given for every five to 7 years after the initial vaccination. Infants may also be vaccinated this way. However, there have been some rare reports of adverse reactions to yeast derived vaccines. Some possible adverse reactions include skin, rheumatic, vasculitic, ophthalmologic, hematological and neurological reactions (Grotto et al., 1998). The immunogenicity and protective efficacy of hepatitis B vaccination is directly related to the levels of anti HBs titers of greater than 10mIU/ml after a primary vaccination series. The vaccines produced by each manufacturer have to be evaluated in clinical trials to determine the age specific dose that achieves the maximum seroprotection rate.
Passive immunization:

HBIG is prepared by the Cohn Oncly fractionation procedure from the serum containing high titers of anti HBs and is standardized to 100,000 IU of anti HBs/ml. HBIG is effective often in combination with Hepatitis B vaccine, as post exposure prophylaxis following a) Perinatal exposure for an infant born to an HBsAg positive mother. b) Percutaneous or mucous membrane exposure to an HBsAg positive blood or in blood. c) Sexual exposure to an HBsAg positive person (Beasley et al., 1983; Grady et al., 1978; Redeker et al., 1975).

2.14. Treatment of Chronic Hepatitis B virus infection:

There is no specific treatment currently available for chronic hepatitis B virus infection. Many antiviral agents have been investigated as candidates for chronic Hepatitis B virus infection. In 1976, two studies one with leukocyte interferon and one with β-interferon have suggested that it can affect the serologic profile of Chronic HBV infection (Desmyter et al., 1976; Greenberg et al., 1976). The most promising anti-viral agent is α-2b interferon, which has been licensed by the Food and Drug Administration. The goals for treatment are three folds. They are to eliminate infectivity and transmission of HBV to others, to arrest the
progression of liver disease, improve the clinical prognosis and to prevent the development of hepatocellular carcinoma.

But currently, interferon treatment is the method of choice of treatment for chronic Hepatitis B. The recommended regimen is either 5 million units daily or 10 million units three times a week; given subcutaneously for 4 months (Mahoney et al., 1999). However, patients who are positive for HBsAg and negative for HBeAg and DNA will not likely to benefit from interferon treatment.

Nucleoside Analogues:

First generation nucleoside analogues like Vidarabine, Acyclovir, Ganciclovir, Zidovudine, Ribavirin, Didanosine and Zalcitabine did not effectively suppress viral replication and had serious side effects. The second generation nucleoside analogues like Fialuridine, Lamivudine, Famciclovir, Lobucavir, Adefovir (nucleotide analogue), BMS 200475 and Emtricitabine were more effective in treatment. Fialuridine, a fluoro-iodo-arabinofuranosyl-uracil nucleoside, markedly suppressed HBV DNA (Paar et al., 1992; Fried et al., 1992) but caused multisystem toxicity due to mitochondrial dysfunction in patients treated for more than two months (McKenzie et al., 1995).

Lamivudine therapy is being studied as a possible method of treatment for chronically infected hepatitis B virus carriers. So far, evidences show a tolerated
and sustained suppression of HBV replication during treatment. There also appears to be a decrease in e antigen and HBs production in patients treated thus far (Nevens et al., 1997). In-patients receiving long-term therapy with Lamivudine, resistance has been noted in the form of mutations at the YMDD locus (Leung et al., 1998; Niesters et al., 1998; Fu and Cheng, 1998; Atkins et al., 1998; Honkoop et al., 1997). The best mutation described so far is the substitution of valine or isoleucine for methionine at residue 552.

**Lobucavir:**

Lobucavir is a guanosine nucleoside analogue with activity against many viruses. In a study by Heathcote et al., (1998), in 81 patients treated with Lobucavir for 12 weeks at doses of 200 to 800 mg, there was suppression of HBV DNA than in those who received placebo (P<0.001). Extended course of treatment with Lobucavir was found safe and efficacious (Bloomer et al., 1998). The major side effects included mild anorexia, dizziness, and abdominal pain. The manufacturer recently halted the clinical testing of Lobucavir because of concerns about a possible association between long-term administration and neoplasia in mice and rats.

**Famciclovir:**

Famciclovir, an acyclic guanine derivative, which also inhibits HBV DNA polymerase (Bartholomeusz et al., 1997; Trepo et al., 1996; Main et al., 1996). In
a preliminary trial, Famiciclovir, given 500 mg three times daily, suppressed HBV DNA in all patients and resulted in HBeAg seroconversion in a small minority of patients (Main et al., 1996). Famiciclovir resistance (Bartholomeusz et al., 1997) is associated with mutations at residue 528 (domain B) of the HBV DNA polymerase. This is the reason for Famiciclovir to overcome Lamivudine resistance and for this reason, it is less attractive than other nucleoside analogues.

Adefovir:

Adefovir is an adenine nucleotide analogue that is administered as the prodrug Adefovir dipivoxil. Clinical trials (Gilson et al., 1996; Bloomer et al., 1997; Jeffers et al., 1998; Gilson et al., 1998), have suggested that Adefovir may be effective as first line monotherapy for the treatment of chronic HBV infection. In studies where 12 weeks of Adefovir treatment at daily doses of 30 mg or greater resulted in reduction of 4 log copies in levels of HBV DNA ($P<0.001$) compared with controls. Levels of HBV DNA returned to baseline after treatment in patients who did not seroconvert. Although Adefovir is well tolerated, the development of renal injury in patients treated with doses of 30 mg and higher suggests that a cautious approach towards adverse side effects are needed.

Immunomodulator therapy:

Immunomodulators are non HBV specific and HBV specific. Non HBV specific immunomodulators include Thymosin, Interleukin-2, Interleukin-12 and
Levamisole. HBV specific immunomodulators include preS or S peptide vaccination, cytotoxic lymphocyte epitope vaccination and DNA vaccination (Malik, 2000). Treatment with thymosin for 6-12 months has been associated with a greater HBV DNA and HBeAg response (Mutchnik et al., 1991; Andreone et al., 1996; Chien et al., 1998) mutations have also been reported after thymosin treatment (Tang et al., 1998). Large scale randomised controlled studies in humans are to be conducted.

**Combination therapy:**

Combination therapy as in the case of HIV treatment may yield a better response. Combination therapy should include an antiviral, which will reduce the viral load, an immunomodulator, which might eliminate residual intracellular virus, and therapeutic immunisation, which will induce the loss of the carrier state. As on date combination therapy (Perillo et al., 1990) like the one conducted in United States and Canada with Lamivudine and Interferon did not show improvement in patients who did not respond to treatment with Interferon alone. Although the initial results are not very promising, such combination therapies could hold an answer for effective treatment of chronic HBV infection.

Although interferon therapy may be used in a selected group of patients, it is a short-term therapy and it does not rule out the use of Lamivudine if seroconversion doesn't occur. Although Lamivudine can be used for all patients
with HBV infection who have active viral replication, the drawback to nucleoside analogue monotherapy is the development of mutated HBV strains that are resistant to these drugs.

2.15. Hepatitis C virus (HCV), the causative agent:

HCV is a small, enveloped RNA virus belonging to the family Flaviviridae, which is better understood by modern molecular biology than by conventional virology. Humans are the only known host of HCV, but the virus can be transmitted experimentally to chimpanzees. HCV can be classified into six major genotypes or clades (designated 1-6) and ~100 serotypes (designated a, b, c etc) based on the sequence heterogeneity of the genome. Genotypes 1-3 have a worldwide distribution, genotypes 4 and 5 are found principally in Africa and genotype 6 is distributed primarily in Asia. Although only one species of HCV is recognised at present, multiple infections with HCV has been observed in humans and experimentally reproduced in chimpanzees. Infection can be detected by tests for antibody and viral genomic RNA in serum. However, genetically and serologically heterogeneous HCV strains may not be detected with equal sensitivity by current diagnostic tests. It is confirmed as the major agent occurring parenterally transmitted NANB resulting in both acute and chronic liver diseases, conveniently termed as hepatitis C. While 80% of Acute viral hepatitis C is
insidious, more than 50% of acute HCV infection progress on to chronic liver
diseases including cirrhosis of liver and hepatocellular carcinoma.

2.16. Hepatitis C, the global epidemiology:

Hepatitis C has emerged as a global health problem today. Although
representative prevalence data are not available from many countries, data taken
from published studies and/or data submitted to WHO by 130 countries indicate
that more than 3% of the world's population are infected with HCV. Worldwide,
the estimated number of chronic carriers of HCV remains at more than 170 million
people (WHO, 1997). Most populations in Africa, the Americas, Europe and
South East Asia have anti HCV prevalence rates under 2.5%. Prevalence rates for
the Western Pacific regions average 2.5-4.9%. In the Middle East, the prevalence
of antibody to HCV (anti HCV) ranges from 1% to more than 12%(WHO, 1999).

2.17. Epidemiology of Hepatitis C in the Asian Region:

While reports on hepatitis A and B are available from Bangladesh, those on
hepatitis C are extremely limited. Khan et al., (1993) in a limited study have
shown 2.4% anti HCV positivity in professional blood donors and 0% in voluntary
blood donors and the same for Hepatitis B surface antigen (HBsAg) was 29% and
2.4% respectively. Zaman et al., (1995) have reported 45.3% of the primary
hepatocellular carcinoma to be positive for HCV antibodies and 36% for HBsAg while total Hepatitis B virus marker positivity was 82.6%. As per WHO weekly Epidemiological Record, (1997), HCV prevalence in the general population of Bangladesh is 2.4%.

Studies on Hepatitis C are limited in Bhutan also except for the report by WHO in its Weekly Epidemiological record, (1997) and the WHO, SEARO publication, (1999). The HCV prevalence in the general population of Bhutan according to these reports is 1.30%.

The prevalence of HCV infection in general population (represented by Voluntary blood donors & pregnant women) from all the regions of India ranges from 0.09 - 2.5 % (mean ± SD: 1.31 ± 2.22); However, the commercial blood donors had anti HCV positivity range of 9.4-15.9% (mean ±SD 12.8 ± 3.25) The WHO weekly Epidemiological Record (1997) places HCV prevalence in the general population of India at 1.85%. The data on HCV in acute and chronic liver diseases in different parts of India is as follows. In Sporadic acute viral hepatitis cases, HCV positivity has ranged between 0.2% and 17.6% (mean ± SD: 8.87 ± 6.38) while the same in fulminant hepatic failure (FHF) and Subacute hepatic failure are 6.2 - 45% (mean ± SD: 24.81±18.08) and 23.8-47.0% (mean ± SD: 39.22 ± 10.52) respectively. Hepatitis C is emerging as an important cause of chronic liver diseases among Indian patients [chronic active hepatitis: 15.3-48.5
(mean ± SD: 30.08 ± 15.13); chronic liver diseases: 5.3 - 26.0% (mean ± SD: 16.84 ± 6.54); cirrhosis 3.3 - 31.5% (mean ± SD: 14.4 ± 9.38) and Hepatocellular carcinoma 15.1 - 42.0% (mean ± SD: 28.55 ± 19.02)]. The major risk groups in India acquiring HCV infection are thalassemia patients, renal failure cases undergoing hemodialysis and health care workers. Data on injecting drug users is grossly limited except from South India (56.2%). Thalassemia patients had HCV positivity range of 11.1-36.4% (mean: 19.61%) and the same for renal failure cases was 12.1-45.2% (mean: 30.81%). The occupational risk of HCV among health care workers in India is 1.3-8.8% (mean: 3.56%) (Arankalle et al., 1998).

The WHO (WHO, 1997; WHO, 1999) has estimated HCV prevalence in Indonesia as 2.1 to 2.5%. Reports based on surveys in the general population and voluntary blood donors of Indonesian provinces (Sulaiman et al 1995; Akbar et al., 1997; Darmadi et al., 1996) project HCV carrier status ranging from 2.1 to 3.9% (mean: 2.5%).

HCV studies from Maldives are again scanty and the available data thro' WHO SEARO report (1999)(WHO, 99) projects HCV positivity in the general population of Maldives as 1.8%. No report is available on HCV related liver diseases and about its involvement in high-risk groups. Myanmar is another Southeast Asian country from where HCV data is limited. As per WHO-SEARO
report, 1999 and (WHO, 99), the anti HCV positivity in voluntary blood donors of Myanmar is 3.9% and in hepatocellular carcinoma is 35%.

Based on the available reports prevalence of HCV infection in the general population of Nepal ranges from 0.1-3.4% (WHO, 97; Shrestha et al., 1994; Nakashima et al., 1995; Shrestha et al., 1996) (mean ± SD 1.11 ± 1.54), 1.3% in AVH patients, (Shrestha et al., 1996) 8.0 % among chronic liver diseases, (Shrestha et al 1994) 60.0 - 94.0 % (mean ± SD 78.16 ± 17.11) in injecting drug users (IDU) (Shrestha et al., 1996; Shreshtha et al., 1997; Shrestha et al., 1998) and 5.0% in haemodialysis cases.(Shreshtha et al., 1997).

Park et al., (1995) have reported HCV prevalence in the General population of Korea to be 1.6%; 53.1% in chronic hepatitis, 30.5% in patients with cirrhosis of liver and 29.0% in HCC cases. However, Shin et al., (1996) have shown 30.3% HCV positivity in HCC while Lee et al (1997) reported a lower HCV positivity (9.3%) in HCC patients of Korea. Lee et al., (1996) found 15% of their hemodialysis patients to have been infected with HCV. Of the limited literature available, HCV in Sri Lankan general population is reported to be 1.4% (WHO, SEARO; WHO, 1999), while no report is available on its role in acute and chronic liver diseases besides high risk groups from Sri Lanka. De silva et al., (1994), have reported 14.9% HCV positivity in alcoholic cirrhosis cases.
In Thailand, the general population has HCV infection ranging between 0.5 and 5.6% (mean ± SD: 2.94 ± 2.15) with the commercial sex workers having a higher risk of acquiring HCV infection (9.5%). In the liver diseases group, 23.0% of CLD patients and 8.4-17.0% of HCC cases were (mean ± SD 12.23 ± 4.37) due to HCV. The problem of HCV is implicated in 20% of hemodialysis cases (Leungrojanakul et al., 1994), 23.8% of thalassemics (Laosompat et al., 1997) and 95.3% of injecting drug users. (Luksamijarulkul et al., 1996)

2.18. Modes of transmission of HCV

Most risk factors associated with transmission of HCV in the United States and other developed countries were identified in case-control studies conducted during 1978-1986 (Alter et al., 1982; Alter et al., 1989). In developed countries, these include (WHO, 1999) a) previously unscreened blood, blood products and organs; b) intravenous drug users; c) individuals undergoing chronic haemodialysis; d) health care workers with percutaneous exposure from contaminated needles or sharps; e) persons who participate in high risk sexual practices; f) persons undergoing medical or dental procedures with inadequately sterilised instruments. Sexual and household transmission are uncommon.

In developing countries, the primary sources of HCV infection include (WHO, 1999) (a) transfusion of blood or blood products from unscreened donors:
(b) transfusion of blood products that have not undergone viral inactivation; (c) chronic haemodialysis; (d) parenteral exposure to blood through the use of contaminated or inadequately sterilised instruments and needles used in medical and dental procedures; (e) the use of unsterilised objects for rituals (e.g. circumcision, scarification), traditional medicine (e.g. Blood letting) or other activities that break the skin (e.g. tattooing, ear or body piercing); (f) intravenous drug abuse and (g) household or sexual contact of HCV infected persons though marginally.

Mother to infant transmission of HCV has been observed globally, but the risk is probably less than 5% unless the mother is co-infected with human immunodeficiency virus (HIV) (WHO, 1999). There is no association between transmission and the type of delivery (caesarian section or vaginal delivery) and no association with maternal breast-feeding. Infected infants progress to chronic disease with a benign course, at least initially. The long-term outlook is not known.

While there are differences in the relative importance of the various modes of HCV transmission in developed and developing countries, and epidemiological studies can better define the local situation within a country. The following settings have accounted for the majority of HCV infections world wide:
1) Transfusion of unscreened blood and blood products

2) Nosocomial transmission during medical and dental procedures from inadequately sterilised instruments, poor infection control practices and unsafe injections.

3) Intravenous drug use

4) Chronic haemodialysis and

5) High risk sexual activity (multiple sex partners)

2.19. Screening and Diagnostic Tests

Serologic Assays

The only tests currently approved by the U.S. Food and Drug Administration (FDA) for diagnosis of HCV infection are those that measure anti-HCV (Gretch, 1997). These tests detect anti-HCV in ≥ 97% of infected patients, but do not distinguish between acute, chronic, or resolved infection. As with any screening test, positive predictive value of enzyme immunoassay (EIA) for anti-HCV varies depending on prevalence of infection in the population and is low in populations with an HCV infection prevalence of <10% (MMWR, 1991; Kleinman et al., 1992). Supplemental testing with a more specific assay (i.e.) recombinant immunoblot assay [RIBA] of a specimen with a positive EIA result prevents reporting of false-positive results, particularly in settings where asymptomatic persons are being tested.
Supplemental test results might be reported as positive, negative, or indeterminate. An anti-HCV positive person is defined as one whose serologic results are EIA test positive and supplemental test positive. Persons with a negative EIA test result or a positive EIA and a negative supplemental test result are considered uninfected, unless other evidence exists to indicate HCV infection (e.g. Abnormal ALT levels in immunocompromised persons or persons with on other etiology for their liver disease). Indeterminate supplemental test results have been observed in recently infected persons who are in the process of seroconversion, as well as in persons chronically infected with HCV. Indeterminate anti-HCV results also might indicate a false-positive result, particularly in those persons at low risk for HCV infection.

2.20. Nucleic Acid Detection

The diagnosis of HCV infection can also be made by qualitatively detecting HCV RNA using gene amplification techniques (e.g. RT-PCR) (Gretch et al., 1995). HCV RNA can be detected in serum or plasma within 1-2 weeks after exposure to the virus and weeks before the onset of alanine aminotransferase (ALT) elevations or the appearance of anti-HCV. Rarely, detection of HCV RNA might be the only evidence of HCV infection. Although RT-PCR assay kits for HCV RNA are available for research purposes from various manufacturers of
diagnostic reagents, none has been approved by the FDA. In addition, numerous laboratories perform RT-PCR using in-house laboratory methods and reagents.

Although not FDA approved, RT-PCR assays for HCV infection are used commonly in clinical practice. Most RT-PCR assays have a lower limit of detection of 100-1,000 viral genome copies/ml. With adequate optimization of RT-PCR assays, 75%-85% of persons who are anti-HCV positive and >95% of persons with acute or chronic hepatitis C will test positive for HCV RNA. Some HCV infected persons might be only intermittently HCV RNA positive, particularly those with acute hepatitis C or with end stage liver disease caused by hepatitis C. To minimize false-negative results, serum must be separated from cellular components within 2-4 hours after collection, and preferably stored frozen at -20°C or -70°C (Davis et al., 1994). If shipping is required, frozen samples should be protected from thawing. Because of assay variability, rigorous quality assurance and control should be in place in clinical laboratories performing this assay, and proficiency testing is recommended.

Quantitative assays for measuring the concentration (titer) of HCV RNA have been developed and are available from commercial laboratories (Roth et al., 1996), including a quantitative RT-PCR (Amplicor HCV Monitor™, Roche Molecular Systems, Branchburg, New Jersey) and a branched DNA (deoxyribonucleic acid) signal amplification assay (Quantiplex™ HCV RNA
Assay (bDNA), Chiron Corp., Emeryville, California). These assays also are not FDA approved, and compared with qualitative RT-PCR assays are less sensitive with lower limits of detection of 500 viral genome copies/ml for the Amplicor HCV Monitor™ to 200,000 genome equivalents/ml for the Quantiplex™ HCV RNA Assay (Pawlotsky, 1997). In addition, they each use a different standard, which precludes direct comparisons between the two assays. Quantitative assays should not be used as a primary test to confirm or exclude diagnosis of HCV infection or to monitor the endpoint of treatment. Patients with chronic hepatitis C generally circulate virus at levels of \(10^5\)–\(10^7\) genome copies/ml. Testing for level of HCV RNA might help to predict likelihood of response to antiviral therapy, although sequential measurement of HCV RNA levels has not proven useful in managing patients with hepatitis C.

2.21. Genotyping

At least, six different genotypes and >100 subtypes of HCV exist (Bukh et al., 1995). Approximately 70% of HCV infected persons in the United States are infected with genotype 1, with frequency of subtype 1a predominating over subtype 1b. Different nucleic acid detection methods are available commercially to group isolates of HCV, based on genotypes and subtypes (Lau et al., 1995). Evidence is limited regarding differences in clinical features, disease outcome, or progression to cirrhosis or hepatocellular carcinoma (HCC) among persons with
different genotypes. However, differences do exist in responses to antiviral therapy according to HCV genotype. Rate of response in patients infected with genotype 1 are substantially lower than in patients with other genotypes, and treatment regimens might differ based on the genotype. Thus, genotyping might be warranted among persons with chronic hepatitis C who are being considered for antiviral therapy.

2.22. Clinical Features and Natural History

Acute HCV Infection

Persons with acute HCV infection typically are either asymptomatic or have a mild clinical illness; 60%-70% have no discernible symptoms; 20%-30% might have jaundice; and 10%-20% might have nonspecific symptoms (e.g. anorexia, malaise or abdominal pain) (Hollinger et al., 1991; Kortez et al., 1993a). Clinical illness in patients with acute hepatitis C who seek medical care is similar to that of other types of viral hepatitis, and serologic testing is necessary to determine the etiology of hepatitis in an individual patient. In ≥20% of these patients, onset of symptoms might precede anti-HCV seroconversion. Average time period from exposure to symptom onset is 6-7 weeks (Kortez et al., 1993b; Marranconi et al., 1992; Seeff, 1991) whereas average time period from exposure to seroconversion is 8-9 weeks (Hollinger et al., 1991). Anti-HCV can be detected in 80% of patients within 15 weeks after exposure, in ≥90% within 5
months after exposure, and in ≥ 97% by 6 months after exposure (Hollinger et al., 1991). Rarely, seroconversion might be delayed until 9 months after exposure (Alter et al., 1992; Alter, 1993; Ridzon et al., 1997).

The course of acute hepatitis C is variable, although elevations in serum ALT levels often in a fluctuating pattern, are its most characteristic feature. Normalization of ALT levels might occur and suggests full recovery, but this is frequently followed by ALT elevations that indicate progression to chronic disease. Fulminant hepatic failure following acute hepatitis C is rare (Liang et al., 1993). However, in developing country especially India, HCV in FHF was reported significantly (Tandon et al., 1991; Panigrahi et al., 1994; Irshad et al., 1995).

**Chronic HCV Infection**

After acute infection, 15%-25% of persons appear to resolve their infection without sequelae as defined by sustained absence of HCV RNA in serum and normalization of ALT levels (Shakil et al., 1995). Chronic HCV infection develops in most persons (75%-85%), with persistent or fluctuating ALT elevations indicating active liver disease developing in 60%-70% of chronically infected persons (Kortez et al., 1993; Alter et al., 1992; Liang et al., 1993; Shakil et al., 1995; Esteban et al., 1991; Seeff et al., 1992). In the remaining 30%-40% of chronically infected persons, ALT levels are normal. No clinical or
epidemiologic features among patients with acute infection have been found to be predictive of either persistent infection or chronic liver disease. Moreover, various ALT patterns have been observed in these patients during follow-up, and patients might have prolonged periods (≥ 12 months) of normal ALT activity even though they have histologically confirmed chronic hepatitis (Alter et al., 1992). Thus a single ALT determination cannot be used to exclude ongoing hepatic injury, and long term follow-up of patients with HCV infection is required to determine their clinical outcome or prognosis.

The course of chronic liver disease is usually insidious, progressing at a slow rate without symptoms or physical signs in the majority of patients during the first two or more decades after infection. Frequently, chronic hepatitis C is not recognised until asymptomatic persons are identified as HCV positive during blood donor screening or elevated ALT levels are detected during routine physical examinations. Most studies have reported that cirrhosis develops in 10%-20% of persons with chronic hepatitis C over a period of 20-30 years, and HCC in 1%-5%, with striking geographic variations in rates of this disease (Seeff et al., 1992; Kiyosawa et al., 1990; Di Bisceglie et al., 1991a, Fattovich et al., 1997; Di Bisceglie et al., 1991b).
Although factors predicting severity of liver disease have not been well defined, recent data indicate that increased alcohol intake, being aged >40 years at infection, and being male are associated with more severe liver disease (Poynard et al., 1997). In particular, among persons with alcoholic liver disease and HCV infection, liver disease progresses more rapidly; among those with cirrhosis, a higher risk for development of HCC exists (Koff and Dienstag, 1995). Furthermore, even intake of moderate amounts (>10 g/day) of alcohol in-patients with chronic hepatitis C might enhance disease progression. More severe liver injury observed in persons with alcoholic liver disease and HCV infection possibly is attributable to alcohol induced enhancement of viral replication or increased susceptibility of cells to viral injury.

2.23. Extra hepatic manifestations in Hepatitis C:

Extrahepatic manifestations of chronic HCV infection are considered to be of immunologic origin and include cryoglobulinemia, membranoproliferative glomerulonephritis, and porphyria cutanea tarda (Koff and Dienstag, 1995). Other extrahepatic conditions have been reported, but definitive associations of these conditions with HCV infection have not been established. These include seronegative arthritis, sjogren syndrome, autoimmune thyroiditis, lichen planus, Mooren corneal ulcers, idiopathic pulmonary fibrosis (Hamman-Rich syndrome), polyarteritis nodosa, aplastic anemia, and B-cell lymphomas.
With the projected 3% HCV infection rate in the world to a tune of 170 millions, an estimated 4 million HCV infected people are in the USA and 5 millions in the Western Europe (WHO, 1999). Based on the analysis made in this report, about 25 million people in the South east Asian countries are infected with HCV with 12 millions in India alone.

**Burden of HCV infection in the general population of Asian countries: a projection:**

<table>
<thead>
<tr>
<th>Countries</th>
<th>Population by 2000 (in million)</th>
<th>Mean HCV carrier status (%)</th>
<th>Projected number infected (in millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangladesh</td>
<td>128.31</td>
<td>2.4</td>
<td>3.08</td>
</tr>
<tr>
<td>Bhutan</td>
<td>2.03</td>
<td>1.3</td>
<td>0.03</td>
</tr>
<tr>
<td>India</td>
<td>1006.80</td>
<td>1.2</td>
<td>12.08</td>
</tr>
<tr>
<td>Indonesia</td>
<td>212.56</td>
<td>2.5</td>
<td>5.31</td>
</tr>
<tr>
<td>Maldives</td>
<td>0.30</td>
<td>1.8</td>
<td>0.005</td>
</tr>
<tr>
<td>Myanmar</td>
<td>49.34</td>
<td>3.9</td>
<td>1.92</td>
</tr>
<tr>
<td>Nepal</td>
<td>24.35</td>
<td>1.1</td>
<td>0.26</td>
</tr>
<tr>
<td>North Korea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(DPR)</td>
<td>23.91</td>
<td>1.6</td>
<td>0.38</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>18.82</td>
<td>1.4</td>
<td>0.26</td>
</tr>
<tr>
<td>Thailand</td>
<td>60.49</td>
<td>2.9</td>
<td>1.75</td>
</tr>
<tr>
<td>Total</td>
<td>1526.91</td>
<td></td>
<td>25.075</td>
</tr>
</tbody>
</table>

In Industrialized countries, HCV accounts for 20% of cases of acute hepatitis, 70% of cases of chronic hepatitis, 40% of cases of end stage cirrhosis, 60% of cases with hepatocellular carcinoma and 30% liver transplants. The
present analysis reveals HCV to be responsible for 4.3% of AVH cases; 24.8/39.3% of FHF/SAHF cases; 29.24% of chronic hepatitis; 39.6% of cirrhosis of liver and 31.1% of hepatocellular carcinoma cases in the Asian countries.

Screening with surrogate markers, such as alanine amino transferase (ALT) assay and tests to detect antibodies to hepatitis core antigen (anti HBc), became mandatory in 1988 (Colin et al., 1997) as the viruses responsible for NANB hepatitis had not been identified. With the discovery of the genome of HCV and the subsequent development of several tests, screening for anti HCV through first generation EIA became mandatory in 1990 and since then, second and third generation EIAs have become available. Inspite of all these measures, post transfusion hepatitis infection still occurs. In the background of differing prevalence pattern of hepatitis viral infections and screening practices, the incidence of PTH varies over an order of magnitude in different parts of the world. For example, prospective studies from Spain and UK reveal rates of PTH approximately 10 and 0.5% respectively. Similarly the association of a history of transfusion in patients with chronic liver disease varies widely; in Japan, with high rates of PTH, the association appears to be obviously high whereas in the UK less obvious. In Japan, Kiyosawa et al., (1982) reported that the frequency of a history or previous transfusion in patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma of the NANBH type was 42.8, 37.1 and 15.1% respectively. Similarly in HCV seropositive patients with cirrhosis or
hepatocellular carcinoma, 48 of 119 (40.3%) and 37 of 92 (40.2%) respectively, have received blood transfusions in the previous 9-53 years. However, one of our recently concluded studies at Dr. ALM PGIBMS, University of Madras, reveals a comparable finding. While HCV positivity was 40.4% (49/121) of histologically proven chronic liver diseases who gave history of blood transfusion during the past 10 years, it was only 12.8% (64/497) in those who did not have any blood transfusion in the past.

**Therapy for Hepatitis C**

Treatment is recommended for patients with chronic hepatitis C who are at greatest risk for progression to cirrhosis, as characterised by

- Persistently elevated ALT levels;
- Detectable HCV RNA and
- A liver biopsy indicating either portal or bridging fibrosis or at least moderate degrees of inflammation and necrosis

**2.24. Persons for whom HCV treatment is uncertain:**

- Patients with compensated cirrhosis (without jaundice, ascites, variceal hemorrhage or encephalopathy)
- Patients with persistent ALT elevation but negative for HCV RNA and with less severe histologic changes (ie. no fibrosis and minimal necroinflammatory
changes) (In these patients, progression to cirrhosis is likely to be slow, if at all, therefore, observation and serial measurements of ALT and liver biopsy every 3-5 years is an acceptable alternative to treatment with interferon).

- Patients aged <18 years or < 60 years

Persons for whom HCV treatment is not recommended

- Patients with persistently normal ALT values with negative HCV-RNA
- Patients with advanced cirrhosis who might be at risk for decompensation with therapy
- Patients who are currently drinking excessive amounts of alcohol or who are injecting illegal drugs (treatment should be delayed until these behaviours have been discontinued for ≥ 6 months)
- Patients with major depressive illness, cytopenia, hyperthyroidism, renal transplantation, evidence of autoimmune disease, or who are pregnant.

The current treatment for HCV infection is:

- Current effective therapy for HCV infection is IFN based, with or without other therapeutic agents such as ribavirin. Ribavirin monotherapy is not recommended.
- For the interim, patients with acute hepatitis should receive IFN-α, 3-6 million units (or 9-15 µg) thrice weekly for atleast 6 months until more effective regimens emerge.
The standard treatment for previously untreated (naïve) patients with chronic hepatitis C is IFN-α, 3-6 million units (or 9-15 µg) thrice weekly for 12 months. However, recent data indicate that a regimen of IFN-α and ribavirin for 6 months or IFN-α monotherapy using different schedules and/or higher doses may significantly improve sustained response rates and become preferred options for treatment in the future.

Adverse side effects to IFN and ribavirin are tolerable, but a fatal outcome (suicide, liver failure, and sepsis) has been observed primarily in-patients with cirrhosis. Less severe side-effects occur in less than 10% of the treated patients and include flu-like symptoms, fatigue, thinning of hair, myalgia, bone marrow suppression requiring dose reduction, neuropsychiatric effects, such as depression and autoimmune disease (thyroid). All patients must be carefully monitored by the prescribing doctor for side effects by using appropriate biochemical, haematological and immunological tests. Appropriate medical records should be maintained.

Types of response: Response to treatment of HCV is categorized as follows:

1. Sustained response: Clearance of HCV RNA from the blood and persistent normalization of serum ALT levels observed 6-12 months after therapy has ended (virological and biochemical response).
2. Transient (relapsing) response: Complete virological and biochemical response at end of treatment followed by the re-emergence of virus and/or elevation of the ALT level during follow-up.

3. Breakthrough response: Temporary virological and biochemical response occurring during therapy followed by reappearance of HCV RNA and/or an abnormal ALT level before the end of treatment.

4. Non-response: HCV RNA remains detectable and/or ALT fails to normalize throughout the treatment phase. When a discordant virological and biochemical response occurs, the virological response should take precedence when interpreting the response to therapy.

The following guidelines should be used when monitoring therapy.

1. Assess "early response" at 3 months by evaluating the patient's ALT or HCV RNA response, according to available resources.

2. Assess "end-of-treatment response" by ALT or HCV RNA estimation when therapy is completed.

3. Assess "sustained response" by ALT or HCV RNA estimation 6-12 months after completion of treatment.