Hepatitis is a generalized term used to characterize inflammation of liver. A variety of etiologic agents including viruses, chemicals, parasites, metabolic dysfunction and auto immune processes are implicated in hepatitis. With the advent of science, distinct viruses are implicated in inflammation of the liver. Thus a new terminology for this distinct pathology of liver was coined as viral hepatitis. The most common feature of hepatitis of any origin is yellow discoloration of the skin and conjunctivae, known as jaundice. The jaundice which is also called icterus, refers to yellow discoloration of skin or sclera by the pigment bilirubin. Jaundice always signifies hyperbilirubinemia but only becomes clinically evident when serum bilirubin exceeds 2mg/dl.

2.1 Evidence of the infectious nature of hepatitis

2.1.1 Occurrence in epidemics:

It has long been known that illnesses presented with jaundice occurred in epidemics with long incubation period (Havens et al 1968) Pickles et al (1939) suggested that disease could be transmitted by personal contact with the incubation period of several weeks. Sporadic cases of jaundice are endemic in institution for mentally retarded children. Within a period of 12 years 1,153 cases of infectious hepatitis were acquired by natural contact (Krugman et al 1967). In all these situations fecal oral mode was the most probable route of transmission.

2.1.2 Transmission by human serum

From 1883 onwards, a number of reports came on incidence of disease resembling catarrhal jaundice. The illness was very common amongst the people who had received injection of human serum as prophylactic measure for other diseases like diphtheria or dog bite. The characteristic feature of this disease was the 2-4 months long incubation period. The disease was common amongst German ship yard workers.
vaccinated with human serum and in groups of children who had been
given plasma from patients who suffered with measles, mumps, diabetes
and rheumatoid arthritis. Yellow fever vaccine which contained small
amount of human serum affected nearly 50,000 American soldiers during
World War II. (Editorial 1942, Sawyer et al 1944). Finally it had been
proved that human serum was the vehicle of transmission and sequel to
blood transfusion. Between 1944 and 1950 icterogenic lots of pooled
plasma were used to experimentally induce infection in adult human
volunteers. Comparable studies on oral and injected filtrates of fecal
material showed three to six weeks incubation period and no cross
immunity between the two (Paul et al 1945). High incidence of enicteric
hepatitis was observed in hospital workers patients, doctors, nurses
working in renal hemodialysis clinic and I.V. drug users. Further
evidences of its parenteral mode of transmission was even identified to
be related to infected dental and tattooing instruments (Bond et al 1977).
Clusters of hepatitis were observed in individuals who were amongst the
cross country runner in Sweden and had sustained minor scratches.

2.2 The "Australia Antigen"

A chain of studies started in 1963 when Blumberg screened blood
samples from diverse populations to study inherited polymorphic trait in
different geographic areas of the world (Blumberg et al 1964). In 1965
Blumberg observed a foreign substance uniquely in the blood of an
Australian aborigine, that reacted specifically with an antibody in the
serum of a hemophiliac patient. This was named as Australia antigen
(Blumberg et al 1970, and 1972). The two substances were found to be
identical and specifically linked with hepatitis B (Giles et al 1969,
Krugman et al 1970) and became the first serologic marker for the
diagnosis of hepatitis B. In 1968, Prince observed a similar substance in
the blood of patients with hepatitis B (Prince et al 1968). Subsequent
studies showed that the Australia antigen was prevalent in some African
and Asian populations and among patients with Leukemia, Lepromatous
Leprosy and Down's syndrome (Blumberg et al 1967). The infectious
virus however was not fully identified conclusively (Bayer et al 1968). Different terms like Australia antigen, serum hepatitis antigen (SH antigen), hepatitis agent (HA), hepatitis Associated antigen (HAA) were used for the same entity (McCollum et al 1970). Antibodies reacting with this antigen were described as AA-antibody, HAA antibody and AA/SH antibody.

2.3 Hepatitis B Virus

The Australia antigen or the hepatitis B surface antigen (HBsAg) became a candidate for the hepatitis virus after the observation of Bayer (Bayer et al 1968) that HBsAg rich plasma contained large number of spherical virus like particle, 20 nm in diameter. However, these particles and associated tubular form present in the plasma transmitting hepatitis B virus were of irregular size, lacked, a complex internal structure, apparently lacked nucleic acid and nucleic acid polymerase and could not be grown in tissue culture. In 1970, Dane et al (1970) discovered the complete hepatitis B virion, 42 nm double shelled particle with partially double stranded DNA and DNA polymerase (Kalpan et al 1973, Robin Son et al 1974 a & b) and was named as Dane particles. Electron microscopy demonstrated that the Dane particle consist of 7 nm outer coat and 28 nm internal coat. Further immuno electron microscopy has demonstrated that antibody directed against HBsAg caused aggregation of Dane particle as well as of the spherical and tubular forms. This indicated that all three morphologic structures have a common antigen on the surface named hepatitis B surface antigen. The nature of the Dane particle was further described by the observation that detergent treatment released an internal core (Almedia et al 1971) Electron microscopic studies have shown the presence of core like particles in the nucleus of infected liver cell. Thus it is now known that the core particle is formed in the liver cell nuclei and the outer coat in the cytoplasm of the infected cell. It is then enveloped and released by the cell as the complete Dane particle. The Dane particle has the ability of synthesizing DNA.. Hepatitis B virus infection is the prototype virus of Hepadna viridae family of viruses. The virus primarily infects the hepatocyte but may be detected.
in peripheral blood mononuclear cells and practically every tissue of the human body.

2.3.1 Geographic Distribution

The prevalence of HBsAg depends upon the variety of interrelated historical, behavioral, environmental and other risk factors. Based on these factors, there are high (7-20%), low (0.20%) and intermediate HBsAg prevalence zones (2-7%) (Nishioka et al 1975). The prevalence of chronic HBV infection has been estimated to be 350 million world wide, which is approximately 5% of the total earth's population (Szmuness et al 1978, Sobeslavasky 1978). The frequency of HBV infected individuals varies geographically. In the Western world, the frequency of HBV infected individuals is just 0.2% (Sobeslavsky 1980). On the other hand it ranges from 5-20% in many parts of south east Asia (Hsu et al 1986) Africa (Both et al 1984, Marinier et al 1985) and West Asia (Sherif et al 1985, Ramia et al 1986). In some isolated communities like Alaskan Eskimos (McMohan et al. 1987) and Australian aborigines (Sherlock 1990) the HBV positivity is as high as 45% and 85% respectively. India is grouped under the intermediate zone. It has the second largest pool of about 43 million HBsAg positive subjects, which is next to China in ranking. Chronic HBV infection in general population in India ranges between 2-13%. (Nayak et al 1987, Tandon et al 1986, Roy Chowdhary et al 1989,.Verma et al 1989). In contrast to the Western world where HBV is mainly a disease of adults, (Szmuness 1975, Gust 1982 ) most people in south East Asia, Africa and West Asia get infected in their early life which mostly results in chronic HBV disease. (Stevens et al 1975, Beasley et al 1982). These individuals are infectious to others and stand very high chance to develop cirrhosis and liver cancer (Recommendations of the immunization practices advisory committee, 1990)

2.3.2 Global Distribution of HBV infection

Despite the availability of effective HBV vaccines, new infection is common. In Europe, approximately 1 million people become infected
each year (VanDamme et al. 1995) and 200,000-300,000 infections occur annually in the US (McQuillian et al. 1999). Corresponding data are not available for the endemic regions of Asia and Africa, but with the high prevalence of HBV in these areas, infection rates are likely to be much higher (Morgolis et al. 1991). Areas of high endemicity (prevalence > 8%) accounts for a total of 45% of the global population and include Africa, Asia (east of the Indian subcontinent but excluding Japan), the Pacific Basin, the Amazon Basin, the Arctic Rim, the Asian Republics previously part of the Soviet Republic and parts of the Middle East, Asia Minor and the Caribbean. Additionally, in parts of Eastern Europe, such as Bulgaria, Romania, Albania and Moldavia, the prevalence of HBsAg positivity is 5-10% of the general population. Areas of intermediate endemicity have a prevalence of chronic HBV infection of 20% and account for a total of 43% of the global population. Parts of Southern and Eastern Europe, the Middle East, Japan, Western Asia through the Indian subcontinent, and parts of Central and South America, are regarded as areas of intermediate endemicity. By contrast, chronic HBV affects fewer than 2% of the population of most of western Europe, Australia and North America, that are generally categorized as areas of low endemicity (Morgolis et al. 1991, Maynard 1990). Even within areas, there may be differences in HBV prevalence based on race and ethnicity (Coleman et al. 1998).

2.3.3 Clinical features of HBV infection

Clinical presentation of HBV infection ranges from asymptomatic to acute hepatitis and severe chronic liver disease. The pathologic consequences of the viral infection are unpredictable and the mechanism of liver damage is not fully understood. The virus is not cytolytic and the infection due to hepatitis B virus causes a necro-inflammatory liver disease of variable duration and severity. Approximately 90% of subjects infected with HBV develop a clinical or subclinical self-limiting illness with spontaneous clearance of virus within a few weeks of infection and remaining 5-10% develop a chronic infection (fig. 1). Chronically infected patients with active liver disease are at very high risk of developing
Fig 1: Disease phenotypes in hepatitis B virus infection.
cirrhosis and hepatocellular carcinoma. The immune response to HBV encoded antigens is responsible for virus clearance and pathogenesis of liver disease. It is used in lab diagnosis of HBV infection (Chisari 1984). Generally severity and frequency of clinical disease increases with age. (Beneson et al 1980). Viral infection starts with an acute phase usually of a icteric form of hepatitis, which progresses to chronic hepatitis and liver disease in six months. This phase further progresses to cirrhosis and hepatocellular carcinoma in 5-10 years. Acute phase can be divided into the following different phases (Kaff and Galambos 1987).

(i) Incubation period
(ii) Prodromal or Preicteric stage
(iii) Icteric phase
(iv) Convalescent period

There can be different forms of hepatitis B virus infection which are diagnosed on the basis of clinical biochemical and immunological pattern.

2.3.4 Acute viral hepatitis (AVH)

Clinical and lab investigation pattern of acute viral hepatitis B is nearly similar to other types of viral hepatitis. The symptoms start with lassitude, weakness, nausea, vomiting and loss of appetite, pain in right upper quotient, loss of weight, low grade fever, arthralgia, skin rashes which commonly can be seen in prodrome or pre icteric phase. Icteric phase appears with the dark yellow colored urine due to bilirubinuria, when bilirubin level exceeds 2.0 - 4.0 mg/dl. One to several days later during cholestatic phase pale clay stool, yellow discoloration of mucous membrane, conjunctivae, sclera and skin appears. In HBV related acute hepatitis the icteric phase usually begins within ten days of the initial symptoms (Zuckerman 1965). Physical examination reveals tenderness and palpable liver within a span of 14 - 16 cm. In 5-15% of patients, palmer erythema and spider angioma may also be observed.

Some times, during the first 4-8 weeks, due to extensive
hepatic necrosis severe impairment of hepatic synthetic function, excretory and detoxifying capacity occurs and is known as fulminant hepatitis. It is characterized by sudden onset of high fever, vomiting and jaundice followed by development of hepatic encephalopathy (Saunder et al 1979, Trey et al 1966). Mortality increases with age and survival is very unusual above the age of 45 years (Mosley 1978, Trey and Davidson 1970).

2.3.5 Biochemical assessment of Acute viral hepatitis (AVH)

The serum levels of aspartic and alanine amino transferases (AST and ALT) and bilirubin gets elevated, although the amount of enzyme elevation has little bearing on the degree of liver damage. it does reflect severity of disease in acute cases in a small proportion of patients serum alpha feto protein levels may also be mildly elevated (Kew 1978).

2.3.6 Immunological diagnosis of acute HBV infection

Diagnostic marker of HBV infection is the detection of hepatitis B surface antigen (HBsAg) in blood and body fluids. The most commonly used methods are radio and enzyme immunoassay (Jelg et al 1985), which can be detected 1-2 weeks before the first symptom appears. High titers of early phase gradually declines and reaches to undetectable levels at the end of 12-15 weeks. An early sign for persistence of virus and become chronic infection is failure of HBsAg titers to decline by 50% within 2-3 weeks. Presence of IgM anti-HBc confirms the diagnosis of AVH. In chronic HBV infection, low titers (<10) of IgM anti-HBc are present. It disappears before HBsAg at around 11 weeks and anti HBe becomes detectable after the disappearance of HBsAg. It provides a lifetime immunity.

2.3.7 Chronic viral hepatitis B

In approximately 2-10% adult patients, acute HBV persists to a chronic infection. Age, sex and body’s immune response are shown to
predispose the development of chronic liver disease. The age at which HBV infection is acquired plays an important role in the production of chronic infection. Between 90-100% of neonates exposed to HBV at the time of birth develop chronic HBV infection (Beasley et al. 1981). Whereas only 20-30% of young children and between 5-10% adult become chronically infected (Beasky 1982). The majority of the population studies reveal that chronic HBV infection state is nearly 1.5-2 times more frequent in males than in females. This difference is not found in early years of life. Chronic HBV infection is very common in immuno deficient and immunosuppressed patients such as in cancer, renal dialysis, Down syndrome, lymphoma or leukemia patients.

2.3.8 Clinical features of chronic hepatitis

A fair number of patients with chronic hepatitis B are asymptomatic. Clinical symptoms could be mild and non-specific. The commonest symptoms are nausea, anorexia, weakness, lethargy and arthralgia. The serum bilirubin does not exceed 4 mg%. Serum ALT levels are elevated and can often fluctuate. Serological markers of HBV infection reflect the chronological profile, clinical and biochemical features of chronic hepatitis. Persistence of markers of replication such as HBeAg and HBV DNA etc is usually accompanied by the persistence of disease.

2.3.9 Diagnosis of HBV related chronic hepatitis

Chronic elevation of ALT levels and persistence of HBsAg in the blood for more than six months is an indication of chronic HBV infection.

2.4 Structure of Hepatitis B Virus Genome

HBV belongs to the group of animal viruses known as the hepadna viridae (Robinson et al. 1982). Other members of this group are
the wood chuck hepatitis virus (WHV), the beechy ground squirrel hepatitis virus (GSHV) and the Pekin duck hepatitis B virus (DHBV). All these viruses have a common structure. They are mainly hepatotropic and lead to persistent viral infection. Only HBV and WHV cause chronic active hepatitis and hepatocellular carcinoma. The infectious form of HBV, also known as the Dane particle, is a 42 nm spherical particle surrounded by a 7 nm hepatocyte membrane-derived envelop that contains the hepatitis B surface antigen (HBsAg) (Ramey and McLachlan 1991). Within the envelop is a 28 nm nucleocapsid core that comprises of hepatitis B core antigen (HBcAg). The core of the hepatitis B virus houses the partially double stranded DNA genome and a viral enzyme that exhibits DNA polymerase, reverse transcriptase and RNAse activity. Genome of the hepatitis B virus is comprised of a double stranded DNA with a complete coding (minus) strand paired with an incomplete, non coding (plus) strand. The two DNA strands of the HBV genome are maintained in an open circular conformation by base pairing of a cohesive overlapping region at their 5' ends. (fig:2)

The long coding or L (-) strand is of a fixed length of about 3,200 nucleotides. The short or S(+) stand is of variable length ranging from 50 to 100% of L(-) strand. The position of the 5' ends of the L (-) and S (+) strands are fixed, while the position of the 3' end of the S (+) strand is variable. DNA sequence analysis of the viral genome have identified four primary translational open reading frame in the L (-) strand which encode the HBsAg (S ORF), HBcAg and HBeAg (C ORF), polymerase (P ORF) and HBx (X ORF) (Tiollais et al 1985) are conserved in the sequences of different strain. Moreover, insertion or deletions present in HBV subtypes are always multiples of these allowing conservation of the reading frames (Gilbert et al 1979, Parek et al 1979, Valenguela et al 1980, Ono et al 1983, Fujiiyama et al 1983, Kobayashi and Koike(1984). In contrast, there are no conserved ORF in the S(+) strand transcript. The L (-) strand thus carries all the proteins coding capacity of the virus. The P region overlaps with all the other three. The expression of gene is regulated by four promoter elements PreS1, PreS2, Cp (core promoter) and Xp (x promoter) and 2 enhancer
elements. These transcriptional regulatory elements direct the synthesis of multiple viral transcripts that are approximately 3.5, 2.4, 2.1 and 0.9 Kilo bases in length and correspond to C, P, S and X ORF respectively. All these transcripts co-terminate at an identical polyadenylation site. This polyadenylation site which is differentially utilized using the viral transcription process is composed of a hexa-nucleotide sequence (TATAAA) that is variant of the canonical eukaryotic polyadenylation site. Several studies have identified a post transcriptional regulatory element (PRE) which is very crucial for the transport of unspecified HBV mRNAs from nucleus to the cytoplasm (Huang and Liang 1993, Huang and Yen 1994 and 1995). Recently it has been shown that HBV PRE contains multiple binding site for nuclear protein which are likely to be required for the transport process (Huang et al 1996). The L protein is produced from another upstream in frame AUG codon on a 2-3 kb transcript arising from the S1 promoter 5' to S.

2.4.1 Surface ORF

This contains 3 in frame transcriptional start codon which direct the synthesis of three distinct surface antigen polypeptides that are known as Pre S1 (large), Pre S2 (Middle) and S (major) proteins. These proteins exhibit distinct amino termini and differ with respect to extent of post translational glycosylation (Ramey and McLachlan 1991). (fig:3)

The S protein is referred to as the major surface antigen because it represents approximately 85% of the HBsAg produced by the virus. The PreS1 and PreS2 proteins present ~15% and 1-2% respectively. Preferential production of major surface antigen is because of the different regulation of the two promoter elements. The HBsAg promoter elements are referred to as the PreS1 and PreS2 promoter. PreS1 promoter regulates the expression of a single transcript of 2.4 Kilohbases. The PreS1 promoter mediates expression from multiple transcription initiation sites that are utilized for the production of a group of mRNAs that are ~2.1 Kb in length and encodes middle and small PreS1 promoter exhibit a classical TATA box sequence that mediated
Fig 2: Structure of Hepatitis B virus Genome
Fig 3 - The surface ORF of Hepatitis B virus A) Protein Products of S-ORF B) Structure of a determinant
transcription initiation. The PreS2 promoter contains 2 initiation sites that function in the absence of TATA motif. PreS2 promoter is functionally stronger than the PreS1 promoter which results in an excess production of major surface protein. Therefore, this regulation is critical for synthesizing the appropriate levels of the three forms of surface proteins.

The activity of PreS1 promoter is down regulated in a manner that is dependent upon a 61 bp sequence (n-3160-3221) present within the PreS2 promoter (Raney 1994). Thus three proteins of 409 (128+55+226) 281 (55+226) and 222 amino acids are formed as the translational product of "S" ORF. The large protein is an important component of the complete virion and is supposed to be involved in host cell binding and virus entry (Bulla and Siddiqui 1989, Neurath et al 1986, Pontisso et al 1989). The most important protein of 226 amino acid (HBsAg) forms a hydrophobic protein, with hydrophilic regions at amino acid residue 30 to 74, which is internal and another at residue 120-156 which is exposed on the surface of HBsAg (Pontisso et al 1989). The amino acid region at residue 99-156 amino acid is rich in cystine and forms the "a" determinant, which is common to all HBV types.

2.4.2 Core ORF

It transcripts nucleocapsid protein HBcAg, which consists of 185 amino acid residues plus an additional 29 amino terminal residues (Precore) (Thomas et al 1988). (fig:4) It is involved in capsid formation, packaging of the pregenome reverse transcriptase complex, trafficking of the capsid in the cell and envelopment (Pumpens and Grens 1988). HBcAg is 21 KDa DNA binding phosphoprotein. It is translated from the AUG codon on a terminally redundant 3.5 Kb RNA arising from the core promoter. Several transcripts arise from heterogeneous start sites within this promoter in coding pregenome RNA. Another antigen synthesized from transcript of the core promoter is the HBeAg which is a processed product of a 25 KDa protein translated from the first two AUG codon on the RNA transcript. The precore protein is processed
Fig 4. The Core ORF of Hepatitis B virus A) Protein Products of Core ORF B) Nucleotide sequence of Pre-core region from 1814 to 1900 (Eco R1 site).
by cleavage at variable sites near the carboxy terminal to generate a heterogeneous species of secreted protein ranging from 15-18 KDa in size and collectively known as "e" antigen (Koschel et al 1999). This antigen is not essential for viral production and its function is not known. Maternally derived HBeAg (vertically transmitted) enters the fetal circulation and induces tolerance to HBV in the infant thereby promoting chronic infection (Ou et al 1986).

The core/pre genomic promoter or core promoter (CP) governs the expression of multiple RNA that exhibits 5' heterogeneity which include core antigen, e antigen, polymerase, mRNAs and pre genomic RNA. The expression of these transcripts is regulated by both the viral enhancer element and a negative regulatory element (NRE). The positive regulation of the CP involves multiple transacting cellular elements. The negative regulation may be mediated by a transacting factor.

2.4.3 Polymerase ORF

It overlaps all the other ORFs and encodes the viral enzymes polymerase and reverse transcriptase activity (Milich et al 1990, Bavand and Laub 1988). The polymerase protein is also translated from the 3.5 Kb, from an internal AUG codon. Besides reverse transcription of RNA pre genome, it is required for initiation of first strand cDNA synthesis by serving as a primer for the reverse transcription and virus assembly. The amino terminal region contains the terminal protein which provides the primary function. This is separated by a non essential spacer region. The carboxyl terminal region carries the RNAase H domain which progressively degrade the pre genome RNA from the DNA/RNA hybrid. This hybrid is formed during the synthesis of DNA minus strand.

2.4.4 X ORF

It encodes a 17 KDa protein known as HBX. HBX is derived from a mRNA transcript of about 0.8 Kb arising from the X promoter.
The major product of this RNA is a protein of about 17 KDa translated from the first of three in phase AUGs on the X mRNA. Alternative transcription initiation can yield two additional HBX polypeptides of about 8.6 and 6.0 KDa (Henkler and Koshi 1996). The HBX protein has not been detected in patient sera due to its short half life (Kwee et al 1992). The function of HBX, in virus replication and pathogenicity is not yet clear. This protein activates gene expression via several signaling pathways like ras-raf mitogen activated protein kinase (MAPK), the tumor promoter activated protein kinase C pathways. Its major function is DNA repair within the nucleus possibly by enhancing DNA repeat. It also allows accumulation of mutations resulting in hepatocellular carcinogenesis. HBX also interferes with p53 tumor suppression gene.

2.5 Variants / Mutants of Hepatitis B Virus

HBV replicates via an intermediate RNA stage. Reverse transcriptase is a translation product of the polymerase ORF and has poor proof reading ability resulting in a high rate of nucleotide misincorporation during transcription. In this way mutations are introduced in the HBV genome. It is estimated that at a nucleotide position of HBV 1-3 x 10⁻⁵ mutations occur each year (Sehek et al 1991). This mutation rate is between 10 and 1000 times lower than the RNA viruses, but 10-100 times higher than other DNA viruses. This is due to asymmetrical replication of HBV via reverse transcription of an RNA intermediate (Okamoto et al 1987).

Since HBV infection may persist for many years or indeed decades, mutations will accumulate over time and may become clinically relevant. Mutations in the HBV genome drastically affect the pathogenicity, evasion of vaccine induced or natural immunity, changes in tissue or species tropism and viral persistence (Summers and Manson 1982, Suzuki and Woodfield 1994, Okamoto et al 1992). The application of molecular biology in the study of HBV has revealed several interesting genetic alterations. Changes in viral epitopes could lead to changes in
the viral structure. In simple terms, viral variants are found in natural isolates while the mutant forms emerge, usually under immunological pressure, often of medical origin.

In recent years, a number of atypical isolates of HBV have been described (Hasegawa et al 1994). Two clinically important antigenic variants of HBV are the HBeAg negative phenotype (Hasegawa et al 1994, Lieberman et al 1983, Akahane et al 1990, Brunetto et al 1990) and the HBsAg vaccine escape variant (Carman et al 1989). Other variants with mutation which may alter replication or gene expression have also been described but the functional significance of these variants is not known. The term variant/ mutant is used for viruses with distinct changes in the sequence which alters the aspect of replication ,gene expression or protein function such as reduced expression of HBeAg, altered antigenicity of HBsAg and resistance of the polymerase to nucleoside analogues. The wild type HBV generally implies replicative HBV infection with HBsAg and HBeAg detectable in the serum. The emergence of new hepatitis B virus, which exist with mutations in its normal genome sequences during the course of a natural infection is called a variant (Carman et al 1990. Okamoto et al 1988). The term variant is used as a collective term describing viruses exhibiting any heterogeneity from the wild type virus. They can be further subdivided into:

2.5.1 Genotype: When a certain viral strain differs by more than 8% on the basis of complete HBV genome sequence analysis, it is defined as a genotype (Okamoto et al 1988). There are six genotype of HBV designated from A-F (Norder et al 1992, 1993, 1994 Naumann et al 1993). In genotype F the difference in sequence across the whole genome is up to 14%. Recently one more genotype of HBV has been discovered designated as “G”. The genome of genotype G was shown to have 3248 nucleotide (Stuyver et al 2000).

2.5.2 Subtype: It is defined as a 4% difference in S gene sequence (Norder 1992 b). Such variability has evolved and become stable over
the millennia. Subtype specific epitopes which are geographically related and genotypically linked, are located in the major hydrophilic region. Two pairs of mutually exclusively determinant \( d/y \) and \( w/r \) gives rise to different subtypes. (Magnius and Norder 1995).

In particular a single point mutation \( K122R \) changes subtype "d" to "y". A similar change \( K160R \) converts subtype "w" to "r" (Bancroft et al 1972). The combination of four types, \( d,y,w,r \) and common group specific determinant "a" gives rise to four major subtypes, \( adw/adr/ayr/ayw \). These can be further subdivided antigenically in to \( ayw1, adw2, adw4 \) and \( adw4,adrq- \) and \( adrq+ \) (Okamoto et al 1987).

2.5.3 Mutant: These have just few amino acid or only one amino acid change and have > 98% homology. They alter the aspect of replication, gene expression or protein function such as reduced expression of HBeAg, altered antigenicity of HBsAg and resistance of the polymerase to nucleoside analogue (Wands et al 1984, Carman and Thomas 1992).

2.5.3.1 Mutations of the S gene: Surface Mutants

A single ORF that occupies more than one-third of the HBV genome encodes the three HBsAg-containing polypeptides. Depending upon the transcription initiation site, three proteins of 409 (128+55+226), 281 (55+226) and 226 amino acid can be produced. HBsAg constitute the common carboxy terminus, being translated from the last AUG. The large HBsAg (LHBsAg) contain the hepatocyte binding site (amino acid residue 21-47). These proteins are highly conserved and the amino acid level, with some regions of variability. The domain within pre-S1 (aa 21-47) which is involved in binding to the hepatocyte is highly conserved and is involved in the attachment of HBV to hepatocyte (Pontisso et al 1989, Brunetto et al 1999). It is also essential for virion assembly and affects the secretion of viral particles (Bruss 1997). A role of protective immunity is also suggested by the presence of both B and T cell epitopes (Ganem 1991) Rearrangement or addition mutants have been identified in LHBsAg in chronic HBV.
infection (Gerlich et al 1990). A missense mutation at an initiation codon of MHBsAg which is involved in the HBV virion assembly is found in certain chronic HBV in some Mediterranean countries and in patients with fulminant hepatitis (Geeken et al 1991, Fernholz et al 1991) Based on antibody-peptide binding, chimpanzee inoculation experiments as well as in vitro mutagenesis, a model of the antigenic region of the small hydrophobic envelope protein was developed (Pollicino et al 1997). This contained two loops joined with two disulphide bridges between amino acid cysteines at 124/137 and 139/147 (Carman et al 1990, Brown et al 1984). The "a" determinant is conserved in all HBV genotypes. There is a well documented evidence that the antigenicity of HBsAg is dependent upon the tertiary structure of the protein. The affinity of an anti-HBs positive human serum for a linear peptide (aa 139-147) is higher than a peptide from aa 124-137. If the former peptide is cyclised between the terminal cysteines, affinity is increased (Carman et al 1990). This region, particularly between amino acid 139-147, is highly conserved. (fig:5)

The antiHBs response following infection or immunization is comprised of antibodies which recognize the "a" determinant. Isolated cases of HBV variants in this region which are predicted to escape neutralization by antibody have been described, and cases of HBsAg positivity have been missed because of failure of current serological assays to detect some variant forms of the antigen. Variants which escape neutralization by monoclonal antiHBs have been selected both during immunoprophylaxis of liver transplant recipients and in vaccinees.

Subsequent studies demonstrated that the substitution from glycine to arginine found at position 145 of the "a" determinant was responsible for the loss of HBs antigenicity (Brown et al 1984). These and other HBsAg variants have been found in both vaccinated (Waters et al 1992) and non-vaccinated subjects (Harrison et al 1991). The failure to detect HBsAg reactivity is due to either (a) reduced antigenicity due to a substitution of glycine for arginine at position 145, (b) reduced antigenicity due to insertion of amino acids at position 122/123 at the
Fig 5: A) Protein Products of S-ORF B) Nucleotide sequence of a determinant with known Amino acid mutation
amino-terminal end of the "a" determinant (Yamamoto et al 1994) or (c) reduced expression of HBsAg due to mutations in the pre-S2 region which contains the small S gene promoter, thus possibly affecting transcription of that gene.

Responses to the plasma derived vaccine were studied in 1590 subjects, mostly infants, from two regions of southern Italy, where the prevalence of HBsAg is greater than 5%. (Pollicino et al 1997, Hou et al 1995) forty four of the vaccines became HBsAg positive, 32 with additional markers of HBV replication, in the presence of antiHBs. Sera from several of these cases were investigated further by PCR sequencing of the "a" determinant (Pollicino et al 1997). One case was a child of an HBeAg positive mother who was given hepatitis B hyper-immune globulin (HBIG) at birth and at 1 month and a course of vaccine at 3, 4 and 9 months. Despite immunoprophylaxis, he became chronically infected with HBV. HBsAg present at 11 months and at 5 years (when antiHBs was no longer detectable) showed reduced reactivity with a panel of monoclonal antibodies which were known to bind to the "a" determinant, although binding to HBsAg from the mother was normal with two of the monoclonal antibodies and only slightly reduced with a third. The sample taken from the child at 5 years had a change from glycine to arginine at residue 145 (G145A) (Pollicino et al 1997). The mother had glycine at this position. Other studies of the prevention of mother-infant spread using HBIG and vaccine showed similar results. G145A has been described in similar cases from Singapore, Japan and the USA. (Waters et al 1990, Zanetti et al 1988, Fuji et al 1992) Thus, to summarize there are 3 main types of surface mutations:

(i)Vaccine escape mutants: These are seen in patients who have generally received vaccination against hepatitis B. These patients contract fresh HBV infection despite adequate levels of anti HBs and can develop cirrhosis and hepatocellular carcinoma.

(ii)Immunoglobulin escape mutants: Patients of chronic hepatitis B, who undergo liver transplantation and are subsequently treated with high
titers of hepatitis B immunoglobulin (HBIG) can have resurgence in their HBV infection even in the presence of very high titers of HBIG. Point mutation at 145 amino acid occurs under immune pressure. Chronic infection by this mutant form leads to loss of graft tissue.

(III) Naturally occurring surface mutants: These are naturally occurring mutants of S-gene which develop during the course of chronic HBV infection. These have been recently isolated for the first time in India. The common feature of almost all the types of S mutations have been the association of amino acid substitution in position 145 of the ‘a’ determinant of the S-gene.

Horizontal transmission of a vaccine escape mutant to a vaccinated person has not been proved. although this must be regarded as a potential problem since anti-HBs-positive vaccines may be susceptible to infection. In the Gambian study, emergence of K141E has been documented in children from two villages who were infected horizontally. It is not known whether this strain has become predominant or whether, with a higher rate of vaccination coverage, it may infect more of the children in these villages.

2.5.3.2 Precore and core mutants

The nucleocapsid protein HBcAg consist of 185 amino acid residues plus an additional 29 amino terminal residues (Precore) (Okamoto et al 1992) It is involved in capsid formation, packaging of the pregenome reverse transcriptase, complex trafficking of the capsid in the cell and envelopment (Pumpens and Grens 1999). The most likely target molecule for cytotoxic T cell and natural killer cell are HBcAg and HBeAg (Koschel et al 1999). Mutations in the core promoter, terminator, precore and core gene give rise to change in serology, increase viral replication and more severe disease. Mutations which lead to an HBeAg negative phenotype are A1762T and G1764A change in core promoter region and M0-I2 (Pignatelli et al 1987).

In some patients, particularly those infected at birth or in the early
years of life, there is rapid emergence of the HBeAg negative variant. This virus, along with the HBeAg positive (wild type) strain can be detected in virtually all patients during the latent infection. Why in some patients the virus then emerges to high levels detectable by standard molecular hybridization assay and why the patients then go on to develop further inflammatory liver disease, is currently unknown. Longitudinal studies in patients with chronic infection demonstrated that the precore stop-codon mutant usually emerged during the course of infection around the time of seroconversion from HBeAg +ve to anti HBe+ve state (Carman et al 1992, Okamoto et al 1990).

In contrast, several studies in patients with fulminant hepatic failure showed that the precore mutant was capable of initiating HBV infection (Tur Kaspa et al 1992, Yotsumoto et al 1992). The infectivity of the precore stop codon mutant has been supported by results of in vitro studies (Liang et al 1991, Beasly et al 1997). Till now, 12 different variants of precore mutant have been described, from Mo to M12 (Beasly et al 1997). (fig:6)

<table>
<thead>
<tr>
<th>Mutation</th>
<th>codon</th>
<th>Nucleotide</th>
<th>amino acid</th>
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<tbody>
<tr>
<td>M0</td>
<td>15</td>
<td>CCT to CCC</td>
<td>Pro to Pro</td>
</tr>
<tr>
<td>M1</td>
<td>15</td>
<td>CCT to TCC</td>
<td>Pro to Ser</td>
</tr>
<tr>
<td>M2</td>
<td>28</td>
<td>TGG to TAG</td>
<td>Trp to Stop</td>
</tr>
<tr>
<td>M3</td>
<td>29</td>
<td>GGC to AGC</td>
<td>Gly to Ser</td>
</tr>
<tr>
<td>M4</td>
<td>29</td>
<td>GGC to GAC</td>
<td>Gly to Asp</td>
</tr>
<tr>
<td>M5</td>
<td>11</td>
<td>TCA to TCT</td>
<td>Ser to Ser</td>
</tr>
<tr>
<td>M6</td>
<td>22</td>
<td>CTG to TTG</td>
<td>Leu to Leu</td>
</tr>
<tr>
<td>M8</td>
<td>25</td>
<td>GGG to GAG</td>
<td>Gly to Glu</td>
</tr>
<tr>
<td>M11</td>
<td>1</td>
<td>ATG to TTG</td>
<td>Start to Leu</td>
</tr>
<tr>
<td>M12</td>
<td></td>
<td>Two A insertion at codon 2</td>
<td></td>
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It seems likely that due to the selection pressure, the presence of anti-HBe, all the variants which emerge in the late phase of the infection, have an inability to produce HBeAg. This phenomenon occurs due to the
Fig 6: HBV e-antigen-negative VARIANTS
conversion of codon 28 to a translational stop codon, but in some patients mutations in the initiation codon of the pre-core open reading frame or presence of frame shift mutations may also result in this HBeAg negative phenotype. Support of the concept that the virus emerges because of further mutation of antigenic epitopes. is the recent observation that during this phase of HBeAg negative viremia with liver disease, there is rapid mutation of the core region to a greater extent than is seen in the HBeAg positive viremic patients (Pollicino et al 1997).

In a Chinese study, it has been shown that the same type of precore mutant is generally found in the family contacts of the person (Akarca et al 1994). For example, all family members of the patient who had M1 mutant, were found to have M1 mutant forms. The clustering of the same mutant form in a family provides conclusive evidence of intrafamilial transmission of HBV. In this study by Akarca, 33% of the asymptomatic carriers, 24% of HBeAg positive and 55% of HBeAg negative family members had precore mutant forms (Akarca et al 1994). This shows that a very large number of family contacts have mutant HBV infection and these subjects have the potential of transmitting this infection to others. It is however, not clear which forms are more transmissible and what are the viral loads produced by the various mutant forms.

Another question which had always worried the investigators was, how does the transmission take place in anti HBe positive individuals? Do they form HBeAg in the individual in whom the virus is transmitted or is it the HBV DNA alone which causes disease? It is important to know that the antiHBe variant, the G1896 A precore form, can be transmitted as such. In fact, these strains are now the most common strains of HBV in many parts of the world. It has been recently shown that such transmission could lead to acute hepatitis (Akarca et al 1994). It was interesting to note that anti-HBe could form without seroconversion from HBeAg positive state since HBcAg and HBeAg share the same B cell epitope. Hence, antiHBe could be formed without formation of HBeAg. This situation is commonly seen in the family
contacts of chronic HBV patients. Further, a recent Korean study showed that the contacts of patients who were antiHBe positive, were always antiHBe positive (Mphahlele et al 1997). The study also contradicted the belief that the transmission is less frequent in antiHBe positive individuals. The presence of precore stop codon mutants is associated with a poor response to antiviral treatment with interferon alpha (Kim and Ahn 1993, Pastore et al 1992, Brunetto et al 1993, Guptan et al 1998). Preliminary results suggest that newer potent antiviral lamivudine could be effective in few of these patients only. The clinical significance of these mutants therefore, throws challenge not only due to diagnostic difficulties, but due to the inherent mode of transmission and finally to choose from limited therapeutic options.

2.5.3.3 HBX mutant

HBX, the smallest HBV viral protein with 154 amino acid residues, has been reported to interact with a wide range of cellular proteins among which are transcription activators that are involved in the activation of proto oncogenes and growth control (Schiff et al 1998, Feitelson and Duan 1997). The finding suggests that the role of HBX is in inhibiting cellular processes of protein degradation. When expressed in transgenic mice HBX can induce HCC in certain cell lines. Frequent mutations and deletions have been identified in small HBX protein. A novel class of HBX mutant has recently been identified in liver tissue of Singapore HCC patients. The function of the X gene has not been precisely defined. In vitro studies show that HBxAg transactivates numerous viral sequences and cellular genes (Twu et al 1993, Kekule et al 1993) and seems to inhibits several p53 functions, including the transactivation of a factor involved in nucleotide excision repair (Kekule et al 1993), p53 induced apoptosis (Wang et al 1994) and inhibition of HBV replication (Wang et al 1995)

It interferes with cellular DNA repair mechanisms (Lee et al 1995) and appears to deregulate the cell cycle (Lee et al 1995). Recently, HBxAg has been shown to specifically bind to proteasome complex involved in
proteolytic pathways (Benn and Schneider 1995). HBxAg appears to be of some importance but not central for viral replication and gene expression in vitro (Huang et al 1996 Blum et al 1992) and seems to have self regulatory function, avoiding excessive HBxAg induced transactivation (Nakatake et al 1993). However in-vivo studies in the woodchuck model showed, that HBxAg minus mutants are not infectious (Kaplan et al 1973) Truncated integrated X gene sequences have been associated with the development of HCC (Murakami et al 1994, Chen et al 1993, Takada and Koike 1990).

Several naturally occurring mutations in the X-gene have been described as replication competent HBV genome with a pre-x open reading frame (Wollersheim et al 1998) and a replication competent HBV variant with a fused X-C reading frame, resulting from a single nucleotide insertion in the X-C overlapping region (Loncarevic et al 1990) and X gene deletion mutants in children with post-transfusion hepatitis spanning part of or all of the X gene (Kim et al 1992). Clearly, more studies are needed to define the biological and clinical significance of normal HBxAg as well as of X gene mutations, including their contribution to the HCC development.

2.5.3.4 HBV polymerase mutant

HBV DNA polymerase consists of 832 amino acids and is composed of 4 functional domains (Pocho et al 1989). The amino terminal region (residue 1-178) is involved in priming the viral template, separated by a spacer region of 158 amino acid residues (179-336). DNA polymerase residue (337-680) possesses dual RNA and DNA dependent polymerase activity while RNase H domain (681-832) is located at the C terminal. Unlike 's' gene mutants, mutations in the polymerase catalytic domain do not occur naturally. Dideoxy 3 thiacytidine (3TC), is an effective suppressor of HBV replication. However, long term use of this analogue has led to the emergence of unresponsive HBV strain (Bartholomew et al 1997). Mutation in the catalytic region of DNA polymerase in particular amion acid catalytic
region of DNA polymerase in particular amino acid Isoleucine or valine in place of methionine at amino acid 552 in the conserved Tyr Met Asp Asp (YMDD) motif results in viral resistance to 3TC.

2.6 Significance of HBV mutants

Existence of hepatitis B virus mutant forms in variable frequency have been well recognized in different populations round the world. The significance of these mutant forms is gradually becoming more evident. The emergence of mutant forms of HBV is a slow process. Under the influence of host immune response, selective mutation takes place in the viral genome which helps its survival within the host (Pirecher et al 1990, Zhong 1999). It is known that HBV mutants could alter the course of the natural HBV infection in an unfavorable way such as, development of severe hepatitis (Brunetto et al 1989, Okamoto et al 1996, Carman et al 1990), immune escape phenomenon (Moriyama et al 1991), vaccine failure, increase incidence of fibrosing cholestatic hepatitis in liver transplant recipients (Fang et al 1993), easy sexual transmission, fulminant hepatic failure (Yotsumoto et al 1992), high incidence of delta super infection (Raimondo et al 1991) and a high potential for causing HCC. The two common mutations, found in patients with HBV related chronic liver disease include HBeAg negative precore mutation (nt 1896, G → A, M2 type) and HBsAg negative surface mutation (aa 145, Gly → Arg). The two mutations together constitute nearly 25% of all HBV related CLD patients. The patients infected with surface mutant form seem to have a milder disease but a higher incidence of HCC at a younger age. Interferon alpha 2b therapy has been found to have good initial success in precore mutant infected patients but has a high relapse rate (54%) after termination of the therapy. Presently no therapeutic approach is universally accepted for surface mutant infection.

2.6.1 Prevalence of HBV mutant in asymptotic chronic HBsAg positive subjects.

Naturally occurring S gene mutants are developed during the
course of chronic HBV infection. They can be prevalent in healthy individuals, chronic HBsAg positive subjects, patients with chronic liver disease, HCC and subjects belonging to high risk groups and their contacts.

HBV mutant forms are quite common in chronic HBsAg positive subjects. The frequency has been reported to be between 5-10% in Mediterranean countries like France, Spain and Greece (Bonino et al 1986, Hadziyannis et al 1983). Recently, the frequency of precore mutant forms has been reported to be as high as one fourth of the total HBV infection in Japan (Hemasaki et al 1994). In India, the antiHBe positivity in HBsAg positive subjects is as high as 30-40% (Tur Kaspa et al 1992). However, confirmatory sequencing studies are needed.

2.6.2 Prevalence of HBV mutant in acute viral hepatitis

The immune response during the acute infection involves humoral as well as cellular limbs of the immune system and therefore it is possible that antibody dependent mechanism also contribute to clearance of the virus. This response is directed predominantly to the HBeAg, core and “a” determinants of the surface protein. As a result of this immune process, about 90% of the subjects undergo elimination of HBV after an acute hepatitis illness. Variants of HBV which have lost the ability to produce HBeAg have been detected in low titer during acute HBV infection (Carman et al 1991) but their emergence to become the dominant strain has not been noted.

2.6.3 HBV mutants and fulminant hepatic failure

Fulminant hepatic failure occurs in approximately 0.1 to 0.5% of patients. The pathogenic mechanism for fulminant hepatitis are not clear. In general it is believed to be caused by massive immune mediated lysis of infected hepatocytes. Therefore in many patients with fulminant hepatitis there is no evidence of HBV replication at presentation (Wright 1992). Several recent studies have found that HBV mutations play a role in development of fulminant hepatitis by way of
enhance viral replication or perturbed immune response. The mutants particularly involve core promoter and precore codon 28 region (Linag 1991, Omata 1991, Sato 1995). Fulminant hepatic failure has been observed associated with post transfusion infection with precore defective mutant HBV (Kojima 1991). Recently transfection into hepatoma cells of a fulminant hepatitis led to higher yield of virion, suggesting that HBV mutant with enhances viral replication is an important factor in pathogenesis of fulminant hepatitis (Hasegawa 1994).

2.6.4 Prevalence of HBV mutants in chronic liver disease

In 5-10% of the adults who remain chronically infected with HBV and in majority (90-95%) of the infants born to chronically infected mothers, viremia persists because of failure of induction or activity of cytotoxic (CD8+) T cells (Penna et al 1991). With the progress of time the hepatic activity may increase, presumably reflecting recruitment of new components of the immune system, antigenic variations in the viral proteins, caused by spontaneous mutation, of the viral genome due to transcription errors introduced by the viral reverse transcriptase. The CD4+ lymphocyte response increases to the nucleocapsid protein and seroconversion to "e" antibody is attempted by the body (Tsai et al 1992). In a prospective clinical study 120 serologically characterized and histologically proven HBV related CLD patients were followed up for almost 5 years (Guptan et al 1996). About 25.8% of these patients were found to be infected with either precore or surface mutant forms. Precore mutations were detected in 15% of patients. Around 39% patients with precore mutations had features of chronic hepatitis. There is little information available on the prevalence of surface mutants in CLD patients. These variants were initially seen in vaccinated children born to Italian carrier women (Zanetti et al 1990) This mutant virus is replication competent The same variant with Gly to Arg substitution at amino acid 145 has now been described in Japan and Singapore (Carman et al 1997, Oon et al 1999, Surya et al 1996). It has usually been seen during passive/active or passive immunization in attempts to interrupt neonatal transmission and in patients receiving homografts for
end stage chronic HBV infection (Wallace and Carman 1997). Although it may take decades before the vaccine escape mutant become predominant world over (Wilson et al 1999) Cirrhosis and HCC was more common in the surface mutant (Guptan et al 1996). The surface gene mutants were found in 10.8% patients in the same study. The mutations were present at amino acid 142. Pro $\rightarrow$ Ser and amino acid 145. Gly $\rightarrow$ Arg.

2.6.5 Prevalence of HBV mutant in hepatocellular carcinoma

Persistent HBV infection has been shown to be strongly associated with HCC. Proposed mechanism of HBV associated HCC include insertional mutagenesis (Brechot et al 1980), transactivating role of the hepatitis BX protien (Twu et al 1987, Wollee et al 1988), truncated Pres2/‘s’ gene sequence (Lauer et al 1992). The hepatitis B virus pre s2 / s gene transactivator is generated by 3’ truncation within a defined region of the ‘s’ gene. There have been several reports of nt 1896 mutants of HBV in HCC (Loncarevic et al 1990, Manzin et al 1992, Clementi et al 1993, Hosono et al 1995, Nimanie et al 1996, Fang et al 1998). Studies from Italy and Southern American blacks have reported that prec 1896 mutants were absent in non HCC tissue and present in HCC tissue, but amino acid substitution at prec codon 23 changing lysine to other amino acid was present exclusively in the tumor tissue (Zhong 2000). However studies from China have documented presence of precore mutant in both tumor and non tumor tissue in an integrated form. Missense mutation at nt 1912 and 1913 in the core gene were found in HCC tissue. In the last decade 3’ truncation of the HBV middle surface gene and the large surface protein have been shown to possess transactivating function and may also have oncogenic potential. A total of 17 point mutations were found among the 18 Chinese patients of hepatocellular carcinoma, that had an intact envelope gene. Ten of these mutations were located in the first loop and seven were in the second loop of major hydrophilic region. Interestingly in six of the HCC tissues with envelope mutant were only identified in the tumor tissue where as this identical mutant sequence was not present in the
corresponding non tumorous tissue. Significant number of HCC patients were found to be infected with mutant viruses in India. The surface mutation was present in 43.7% patients (Guptan et al 1996). Many studies in the past have suggested a possible association between surface mutant infection and HCC in the West (Omata et al 1991, Lauer et al 1994, Blum 1995). These were, however, isolated cases.

2.6.6 Transfusion associated hepatitis (TAH) and HBV mutants

The magnitude of transfusion associated hepatitis could be determined by obtaining two variables namely the incidence of TAH after blood transfusion and a potential risk to the population. The risk of transfusion of HBV from HBsAg undetectable blood transfusion is one in 178 (0.6%). The risk of TAH increases with the number of blood units transfused. The incidence of TAH due to HBV has been dramatically reduced to near negligible levels in developed countries (Hepatitis Surveillance Report No. 55. Atlanta, Centers for Disease Control and Prevention 1994, McCullough 1993).

The national average for HBsAg positivity in healthy donor population in India is around 4.7% (Thyagarajan et al 1996). So the risk of transfusing infected blood without testing for HBV could be as high as 1 in 20. The anti HBe positivity in general population is around 15-25% (Thyagarajan et al 1996, Kant and Arora 1996). Of these, around 9-14% blood donors are reported to be HBV DNA positive (Banerjee et al 1993, Saraswat et al 1996). Thus there is indeed potential risk of developing TAH-B from such individuals through transfusion (Saraswat et al 1996). Posttransfusion fulminant hepatitis has been observed associated with precore defective HBV mutant (Kojima et al 1991). Fulminant hepatitis B was developed in eight recipients of blood units without detectable HBsAg on routine screening. Mutations at codon 28 TGG to TAG was commonest.

2.6.7 Prevalence of HBV mutant in general population

Prevalence of surface mutation in general population has
not been studied in great detail. Some investigators have adopted an indirect approach by studying the prevalence of HBV DNA positivity in HBsAg negative blood donors, which ranges from 0.3% to 1.7% in Europe and Asia respectively. In a Canadian study HBV DNA by PCR was detected in HBsAg negative Anti HBC positive individuals. The HBV DNA positivity by molecular hybridization was found to be 9.9% in HBsAg negative voluntary blood donors in India. However, all DNA positive patients did not have high ALT.

2.6.8 Naturally occurring HBV mutant

The emergence of Hepatitis B e antigen negative variant usually occurs during the late phase of chronic infection and is associated with the return of infectivity and inflammatory liver disease. Transmission of this virus, without change to a new host and a new immunological environment may result in fulminant hepatitis. The second variant, because of an amino acid substitution of Arg for Gly at codon 145, has been observed in a significant number of neonates, who acquire infection inspite of active / passive immunization at birth, but not observed in vaccinated children and adults. To date naturally occurring variants have been described in all coding regions and in some regulatory regions of HBV but mutations affecting the major catalytic domains of the polymerase gene, which could alter susceptibility to antiviral nucleoside analogues, were not detected at all (Ogura et al 1999).

2.7 Familial clustering

Presence of two or more members with the marker of HBV infection, together in a family is termed as familial clustering of HBV infection. Clustering of the HBsAg has been reported in families of asymptomatic carriers and patients with a spectrum of liver disease (Blumberg et al 1966, Hadziyannis and Merikas 1970, Berris et al 1973, Irwin et al 1974, Grossman et al 1975, Szmuness et al 1975). Although the exact
pathogenesis of familial aggregation of HBsAg remains unknown, both genetic and environmental factors have been implicated in transmission of HBV within family. Familial aggregation of HBV has been described previously and some studies have shown that HBV subtypes are similar within families, indicating intrafamilial transmission (Grossman et al 1975, Szmuness et al 1973, Feinman et al 1973). Family size plays an important role in clustering phenomenon. There is always a high tendency for HBV carrier children and those with serologic evidence of prior HBV infection to cluster around other HBsAg positive siblings, particularly those over four years of age (Toukan et al 1990).

2.7.1 HBV transmission

HBV is present in the blood, saliva, semen, vaginal secretions, menstrual blood, sweat, breast milk, tears and urine of infected individuals (Boag 1991)

The virus is transmitted efficiently by a number of routes including transmission from an infected mother to her child (perinatal or vertical transmission), or by percutaneous and mucous membrane exposure to infectious blood or other body fluids (horizontal transmission) (Mast and Alter 1993, Alter 1996). Mode of transmission also depends upon the HBV prevalence rate in the general population and amount of HBV DNA present in the source. There can be three distinct zones. (fig:7)

2.7.1.1 Hyperendemic region

In regions of high endemicity, transmission from an infected mother to child is the main mode of transmission, with the lifetime risk of acquiring HBV greater than 60%. (Alter 1996, Maynard 1990). In this region the infection is almost universal. Some part of South East Asia 30-50% of HBsAg positive female of child bearing age are HBeAg positive and serve as an important source of infection to new born. Perinatal transmission rates from HBsAg-positive mothers are as high as 90%. (Margolis et al 1991). Infections are generally acquired early in life, either at or shortly after birth, or early in childhood by exposure to
Fig 7: Components of hepatitis B virus (HBV) infection, that include an infection source, a susceptible host and an established route of transmission.
members of the extended family who may be carriers of HBV (Gust 1996).

2.7.1.2 Intermediate endemic zone

In regions of intermediate endemicity, individuals of all age groups can be infected, although chronic infection is generally caused by transmission during infancy or early childhood. The lifetime risk of infection is 20-60%. (Alter 1996). This includes countries of North Africa, Middle East, part of Southern and Eastern Europe and South America. The HBsAg positivity ranges from 1-5%. In these regions horizontal transmission is the major mode and commonly infection is acquired during the first five years of life.

2.7.1.3 Low prevalence area

In regions of low HBV prevalence, where the lifetime risk of HBV infection is less than 20%, transmission is primarily horizontal (between individuals). Sexual transmission (either homosexual or heterosexual) in high-risk adults is main mode of transmission in Europe and North America (Gust 1996). Needle sharing amongst intravenous drug abusers or occupational exposure to contaminated blood and blood products continues to be important (Lee 1997).

Individuals aged 15-24 years are generally considered to have the highest rate of infection (Zuckerman 1999). This include all developed countries. HBsAg positivity is nearly 0.1% or less. High risk sexual behavior, intravenous drug abuse and general population movements such as population migration from highly endemic area to low prevalence countries are the major mode of HBV transmission. Though pattern of infection may change rapidly with changing social or sexual behavior.

2.7.1.4 Amount of DNA in Different Biological Fluids

The risk of HBV transmission depends upon the amount of
infectious particle present in the source. Though HBV is present in almost all the biological fluids, semen, urine and saliva are the important biological fluids involved in HBV transmission. Since these fluids normally contain leukocytes which is the major source of HBV DNA (Pontisso et al 1985, Lie et al 1983). In a recent study it has been shown that saliva of the infected person has a major contribution in horizontal transmission of HBV (Zhevachevasky et al 2000).

2.7.2. Horizontal transmission

Horizontal transmission is defined as unrelated to recognized perinatal exposure and excluding parenteral and sexual transmission. Horizontal transmission is the predominant mode of transmission in some populations and occurs mostly during childhood (Beasley et al 1982, Davis et al 1989, Matsuo et al 1990). The term "close contact" is used to describe horizontal transmission, especially for those individuals, including grandparents and baby-sitters who are the main care takers in many families in Taiwan and who are often reluctant to receive medical checkups.

Importance of horizontal transmission and development of chronic carrier state has been correlated with the root of infection, type of onset, age of exposure to HBV and genetic factors (Coltorti et al 1978 and 1984, Lindberg et al 1977, Nasrallah et al 1978, Szmuness et al 1973). It is well known that chronic carrier stage occurs in about 90% of cases when infection is transmitted at birth by a HBsAg +ve HBeAg +ve mother and about 10% if infection is acquired in the adult life (Givsti et al 1980). The study by Vegnente et al (1992) has emphasized on importance of horizontal transmission in the outcome of HBV towards chronicity when acquired in childhood. Similarly Tonge et al (1994) had found that vertical transmission accounted for only 5% of HBsAg +ve subjects in the general population where as horizontal transmission account for almost 48% of infection by the age of 10 years. Horizontal transmission of HBV to infants and children has recently become infrequent in many developed countries, mainly because of routine screening of blood for HBV and the general use of disposable needles.
syringes and other instruments in the hospitals.

2.7.3 Vertical transmission

Transmission of HBV from chronically infected mothers to their babies can occur during the prenatal period. The risk of infection in the infants may reach to 90% and appears to be related to ethnic groups. Approximately 22-30% of infants born to HBV positive mothers become infected in early infancy and the transmission rate and outcome of disease depends upon the maternal status of HBeAg/ Anti HB e (Okada et al 1976, Shiraki et al 1980, Stevens et al 1979). Infants who are born to HBeAg +ve mothers are usually asymptomatic who were not infected at birth, but later become HBV positive (Shiraki et al 1977). Perinatal transmission is of intermediate frequency in mothers of West Asian or Afro-Caribbean origin, because of the less frequent e antigen positivity. The infection transmit commonly during first five years of life as a result of horizontal transmission.

Mother to child transmission of HBV depends on the gestational age at the time of maternal hepatitis. Hepatitis in the first to second trimester rarely causes HBV infection of the new born. There is a substantial risk of perinatal infection if the mother has acute hepatitis B particularly during the third trimester of pregnancy or within two months after delivery which suggests that mother to infant transmission of HBV occurs mainly in the perinatal period rather in-utero (Schweitzer et al 1973 ). Intrauterine infections are uncommon since the virus does not cross the intact placenta. The precise mechanism of perinatal infection is uncertain but it probably occurs during or shortly after birth as a result of leakage of maternal blood into child's circulation.

2.7.4 Environmental factors

The frequency of HBV in a subset of population is a function of several factors which increases the risk of viral entry into the body. These extraneous largely environmental and life style related factors

In a recent study saliva was found to be the major source for the HBV transmission (Zhevaachevsky et al. 2000). As a general rule, it was believed since long that HBsAg carrier state is seen more in tropical than in temperate areas (Prince 1970), higher in children and urban communities (Jain et al. 1992) and has an inverse relationship with the socio-economic status (Szmuness et al. 1978). This problem gained special attention in families of chronic liver disease patients with everyday close contact.

2.7.5 Genetic factors

Over the last few years determination of various immune responses to infectious pathogens (Jepson et al. 1997) in the field of human infectious diseases, immunogenetics has developed an unique field concentrating on the complete human genome to identify a large number of immune responsive gene. The key issue is to define immunogenetic polymorphism which determines differential susceptibility to infectious diseases. Genetic contribution could be studied independent of environmental effects from studies of adoptee or of twin. Twin studies are increasingly being used to estimate extent of genetic determination of various immune response to infectious pathogen. Matched qualitative and quantitative differences have been observed in both humoral and cellular immune responses to antigens from these pathogens (Troye-Blomberg et al. 1990, 1991). Tuberculosis has been frequently studied and much higher concordance rates were found in monozygotic than dizygotic twins (Comstock 1978). In a study of leprosy patients, higher disease concordance rates were found in monozygotic twins (Fine 1981). More recently twin studies of hepatitis B virus, Helicobacter pylori infection and malaria have been reported.
The role of host genetics in hepatitis B was studied by segregation analysis and twin studies from China (Haun et al. 1982, Lin et al. 1989). In Chinese twins, higher rates of concordance for hepatitis B virus persistence were found in identical than non-identical twins (Lin et al. 1989). Because of the variation in the environmental factors and geographical distribution of pathogenic strains, it becomes necessary to look into the host genetics and the resulting immune responses.

2.7.5.1 Candidate gene

Association studies of candidate genes in infectious diseases have increased rapidly as more polymorphisms are identified in genes considered to have important role in the pathogenesis or protection. However, it is difficult to interpret genetic association with infectious diseases due to inconsistencies between different populations. Some of this population heterogeneity is genuine but some results from poor study designs.

The genetic background of the host contributes a lot to determine the outcome of HBV infection. The process of identifying the genes responsible for increased or decreased susceptibility to diseases have been successful in other infectious diseases like malaria (Haun et al. 1982) and inflammatory bowel disease. Genome scanning on affected sibling has been carried out though, it has not been applied in HBV. An early study (Lin et al. 1989) have suggested that an autosomal recessive mode of inheritance for persistence of hepatitis B surface antigen carriage state exists. A low level of anti-HBs, (less production) (Larouze et al. 1976) is controlled by the host genetic factors specially in males (London and Drew 1977), who are - 1.5 times more likely to develop chronic HBV infection than females. The plasma disappearance rate for HBsAg is slower in males compared to females (Craxi et al. 1982). Clearance of viral infection is affected by the cellular and humoral immune system. Production of antibodies against envelope of HBV by humoral response plays a major role in the protective immunity against HBV. This is a T-cell dependent process.
2.7 HLA Studies

The major histocompatibility complex is involved in the regulation of the immune response. The class I antigens (HLA-A, B, and C) are mainly recognized by T cells that have the ability to develop in cytotoxic cells. The class II molecules (HLA DR and DQ) play a role in the activation of the regulatory T lymphocytes (Troye – Blomberg et al 1991).

HLA molecules are cell surface glycoproteins that bind and present the peptide to T cells during immune response. Although, each individual HLA allele has the potential to bind many different peptides, there is a degree of specificity which results in different HLA molecules having individual peptide binding receptor. This is widely thought to be the molecular basis for many of the well known associations against chronic infection such as malaria (Miel et al 1991). Elimination of virus essentially depends on the response of CD8+ cells to peptide epitopes presented by MHC class I molecules. The CTL response against the virus is specific to some epitopes. The magnitude of T cell response to different peptide epitopes is variable. It is dominant to some peptides and subdominant to few (Sercarz et al, 1993). In MHC class I pathway, proteasome specificity, transporters associated with antigen processing selectivity, affinity of MHC for different epitopes and epitope dissociation, contributes to outcome of CTL response. Infected hepatocytes with HBV will present antigen to the CD8+ lymphocytes. In acute hepatitis, HBV specific CTL are readily detected in peripheral blood (Bertoletti et al, 1991. Robers et al, 1995). In patients with established chronic infection, CTLs are not readily detected even though CD8+ lymphocytes are present in the liver. To date, however, there is no convincing study which has shown association of class I alleles with outcome of infection. It has been shown that the CD4+ helper cell response directed against the nucleocapsid antigen of HBV is significantly greater in patients with acute self limiting infection (Ferrari et al 1990). Variation in MHC class II allele sequence determines which antigenic peptides are bound and presented to CD4+ T helper cell. Therefore, defective poor antigenic presentation is considered as one of the causes of persistence of HBV infection. A number of studies have
correlated association of different alleles with different clinical situation of HBV infection. Study from Gambia revealed an association of HLA DRB1*1302 with viral clearance (Thursz et al 1995, Hohler et al 1997). Association of HLA DRW 6 with acute infection (Van Hattun et al 1987) and DR 2 / DR 7 with persistent infection (Almarri and Batchelor 1994) has been shown. HLA DRW6 is a serological supertype of HLA DRB1*1302. So an indirect relation can be established between them. All these studies suggest a strong association of MHC class II with recovery from HBV infection (Thursz et al 1997).

Micro satellites are small arrays of tandem repeats which are simple in sequence (1-4bp) and are interspersed throughout the genome. The mononucleotide repeat runs of A and T are very common and together account for ~10 mb or 0.3% of genomic fragment. In case of dinucleotide repeats array of CA repeat (complimentary TG) are very common accounting for 0.5% of genome. These are highly polymorphic. Tri- and tetra nucleotide tandem, repeats are comparatively rare but polymorphic. However the significance of micro satellites is still poorly known. For the first time DNA Polymorphism has been used as marker which are sufficiently numerous and spaced across the entire genome. These developments allow disease gene mapping. The first generation of DNA markers were studied by restriction fragment length polymorphism. Using this technique the whole genome cannot be mapped. The standard tools for linkage analysis are now micro satellite DNA which could present the clear picture of the whole genome.

2.7.6 Viral Factors

Besides the host genetic factors, viral characteristic also contribute to the persistence and outcome of HBV infection. Associated factors are:

2.7.6.1 Viral Load: It has also been shown that patients with chronic HBV infection have a high viral load. However recent reports indicate that this may not always be true. In patients with clinical recovery from
acute hepatitis B, HBV DNA often remains undetectable using PCR. Despite the presence of anti HBs and HBV specific cytotoxic T cells several factors influence the response rate. The most important pretreatment variable in favour of interferon treatment response in adults with chronic HBV are low HBV DNA and high serum ALT levels. (Perillo et al 1990, Wong et al 1993). Although in a study to assess antivirals in HBeAb positive chronic hepatitis, there was no difference in baseline replication level in responder and non responder. (Brunetto et al 1993). Some investigators did not find any difference in response to interferon therapy in patients with HBV DNA levels between 10^ - 10,000 pg/ml

2.7.6.2 Viral Mutation: Viruses which use reverse transcriptase , develops a lot of heterogeneity as this enzyme lacks proof reading function (Rook et al 1987).

The rate of mutation averages 1:1,000, HBV also belongs to this category of viruses. The virus gets mutated under the body's immune pressure to escapes from the host T cell recognition. Most common mutants are precore stop codon mutant which develop due to point mutation of nucleotide G – A at 1896 forming translation stop codon (TGG – TAG) in precore gene. The occurrence of non sense, frame shift or initiation codon in this region can also arrest expression of HBeAg without altering the replication capacity of the virus. Theoretically the reason for the predominance of this mutant could be as long as both e +ve and e-ve DNA share the same epitopes in the core region, HBcAg specific cytotoxic T lymphocytes can not distinguish between the target peptides expressed by two DNA's. Therefore , e minus DNA may be accompanied by additional mutations in the core region, which may affect cytotoxic T lymphocyte recognition. It has been recently shown that mutations in the core region are associated with more severe disease and and a result of antiviral immune pressure. (Moriyama et al 2000, Shindo et al 1999). Fulminant hepatic failure occurs in ~ 0.1% to 0.5% of patients. The pathogenic mechanism for fulminant hepatitis are not clear. Several recent studies have found that HBV mutants specifically, core promoter and precore stop codon mutants are more frequently found in patients with fulminant hepatitis.
2.7.6.3 Viral Genotype: HBV genotypes have a specific geographical distribution. Few recent studies have shown an association of genotypes with severity of disease, development of HCC and outcome of antiviral therapy. Similar to Western world, genotype A and D are prevalent in India whereas Eastern Asia have B and C genotype. In a study from Spain have shown that genotype D is commonly found with acute hepatitis B infection whereas genotype A is present in chronic infection. Also genotype D commonly circulates with precore stop codon 28 type mutant. An association of genotype B with the development of HCC was found in young patients from Taiwan (Chen et al 2000). In an isolated report from West suggest a favorable interferon treatment with genotype A.

2.8 Familial Clustering of HBV Infection possible Mechanism

Epidemiological studies have consistently shown that in the endemic areas, majority of HBV infection occurs in the infancy and early childhood (Tahor et al 1985). While percutaneous exposure to blood, sexual transmission and mother to infant transmission are the most widely known modes, the most prevalent mode of transmission in the endemic regions is the familial spread (Toukan et al 1990). Household contacts of subjects infected with chronic HBV infection are at very high risk from sexual, horizontal, vertical and other unexplained modes. Since HBV is present in many body fluids, the risk of transmission in family members depends on the immunity of the host, the infectivity of the patient, and the nature and duration of exposure. There could be three clinical situations in which HBV could be transmitted to the close contacts: (a) from a chronic HBsAg positive subject, (b) an acute HBV infected patient, (c) a patient with HBV related chronic liver disease, and, (d) HCC patients.

The social practices and living habits of individuals vary from country to country. It is therefore of fundamental importance to investigate the mechanisms of HBV spread in the families of chronic liver disease patients. Different studies suggest different factors responsible for the familial clustering. Toukan et al (1990) have
suggested that the family size and clustering phenomenon are interrelated. Larger families had a significantly greater number of HBV carriers. Greater proximity of members to each other specially among poor, and contribution of family members other than index carrier increases the prevalence rate. The concept of horizontal siblings transmission fits well with socio cultural settings. Mainly in poor, rural children living in large families, small dwellings where many children sleep and eat at close quarters, share utensils and belongings, cuts, grazes and unhealed scars are often left unattended and become the source of infected serum and blood (Al-Faleh et al 1988). Several studies have shown relation to index case as an important deciding factor for clustering. A study from South Africa shows that the degree of relatedness to index patient did not influence the risk of becoming an HBV carrier. Since members of the extended family and unrelated household members had similar risk of being HBV carrier. However male members were more likely to become chronic carriers. This could be because of sex linked defect of T cell function (Karim et al 1991).

A study from Spain (Porres et al 1989) documents intrafamilial spread as high as 33.5%. An interesting finding in this study was the relationship between presence of active replication markers, receptors for polymerised human serum albumin (pHSA-R) in the blood of HBV carrier. The activity of pHSA-R is located in a protein product of the pre S2 region of HBV genome. Its presence is interpreted as high infectivity of the index carrier.

In a recent study from the sub-Saharan African Island, Ghana, the overall prevalence of sero positives (any HBV marker) was 74.7% and the prevalence of HBsAg was 20.9% (Martinson et al 1998). The data suggested that there is a continuous non-uniform acquisition of HBV infection through advancing age. The behaviors most commonly associated with prevalence of HBV infection were sharing of bath towels, sharing of chewing gums, or partly eaten candies, sharing of dental cleaning materials and biting of fingernails with scratching the backs of carriers (Porres et al 1989). In a Korean study on the other hand, drinking from same vessels (relative risk 12.1) was found to have
greater relevance besides sharing the same towel (relative risk 11.5) (Mphahlele et al 1997).

Prevention and control of percutaneous routes has been taken care of by improving the hygienic standards. Hence, the main mode of transmission of HBV today is through the pool of contacts. It is therefore, of vital importance to identify the modes of such transmission.

In a large study from Jordan, 402 (36%) of the 1115 family members studied had exposure to HBV ( Taukan et al 1990). The frequency of exposure increased to 92% in the age group of above 60 years. The infection rate increased from 57% to 98% when the number of carriers increased from 1 to 3 in a family. The risk of HBV was 10% in a mean of all age groups. 50% of sexual partners were also exposed. In one study in Papua New Guinea, South Africa and Sardinia, 5% of the carriers were identified with escape mutants (Pawlotsky et al 1997).

The antiHBe positive form of chronic severe hepatitis seen in Greece and Italy is caused at least in some cases, by the transmission of an A-1896 containing strain, and an HBeAg positive phase is not required (Akarca et al 1994). Genetic segregation analysis of the HBsAg carrier studied in large population supports the hypothesis of Blumberg that chronic carrier state segregates in a pattern consistent with autosomal inheritance. The potential modes for the intrafamilial spread of HBV are numerous since HBsAg is present in most body fluids, including saliva, semen, menstrual discharge, urine and feces. In apparent parenteral inoculation of HBV occur from common personal utensil, razors, tooth brushes etc. Leichtner et al (1981) reported a family in which clustering of HBV infection could be accounted for both horizontal non parenteral transmission of HBV via the exchange of chewing gum contaminated with oral secretions among children (Kashiwagi et al 1984) Szmuners et al (1973) and Coltorti et al (1978) proposed that genetic factors may be responsible for the rate of chronicity in some families since they found that such an outcome was more frequent in blood relatives as compared to non blood relatives.
Many studies have suggested HLA linkage to HBV infection (Forzani et al. 1984, Van Hattum et al. 1987, Vegnente et al. 1988). It has also been suggested that a genetically determined defect in immuno regulation plays a part in chronic infection (Lindberg et al. 1977, Nasrallah et al. 1978, Vegnente et al. 1991).

2.9 HBV in Asia Pacific region:

The prevalence of chronic HBV infection has been estimated as 2.8% in developed countries and 7.6% in developing countries. There is a wide variation of HBV infection in Asia Pacific region. East and Southeast Asia are among the most populous region in the world and made up of countries with different degrees of prosperity and are in different stages of industrialization. Hepatitis B infection is hyper endemic in this region, prevalence varies from 0.5 to 20%. It is estimated to be 4.6% in China, 2.5-9.0% in Indonesia, 0.8% in Japan, 7.3% in Korea, 5-16% in Philippines, 10% in Taiwan and >8% in Thailand and Singapore (Suzuki et al. 1994). The prevalence is low in Australia and New Zealand (<1%). Chronic HBV infection in this region was found to be associated with a significantly increased risk of chronic liver disease (Beasley et al. 1981). More than 75% of the estimated chronic HBV infected subjects live in this region. Many of these infections are acquired early in life. Striking discrepancies in the age pattern of HBV infection has been reported in Asia Pacific region. Wide spread infection occurs in infancy and early childhood in high prevalence countries like Southern China and Taiwan. In low prevalence countries, HBV infection is most common in young adults. The age at which the plateau in the prevalence of HBV infection occurs vary from high to low risk countries. The younger the age, higher the probability of becoming chronically infected (Chen et al. 2000). While broad details of epidemiology of HBV infection can be defined, major differences in the pattern of infection do occur within countries, towns, villages and families (Gust. 1996). Among the most striking are the different infection and carrier rates among ethnic groups living alongside one another. In Fiji chronic HBV infection rate is considerably higher in indigenous
Melanesian population than in the equally numerous Indian population. The trend is also found in the different ethnic groups in Malaysia. The epidemiology of hepatitis B infection in a community can change significantly over a comparatively brief period through public health intervention, changes in the pattern of IV drug users or mass movements of people through migration. The prevalence of infection among children in Taiwan and Singapore has declined dramatically, due to widespread immunization campaign (Chen et al 1991). In these countries, the most important mode of transmission is perinatal. These infections are usually asymptomatic and hence undetected and are responsible for maintaining the chronic infection pool (Gilbert et al 1984, Coursaget et al 1987). Horizontal transmission from family members play a significant role in spread of HBV among siblings in a family (Tan 1991). Percentage of horizontal transmission in China is 80-87%, Korea 80%, Singapore 80%, Taiwan 50-60% and Philippines 40%. The incidence of post transfusion hepatitis B throughout the region is reduced tremendously after introduction of HBsAg screening of donor blood. Other factors are use of disposable needles and immunization programme.

2.10 Indian Scenario

India comes under the intermediate zone for HBV with HBsAg prevalence of 2-7% in general population. Nearly 60% chronic liver disease in India is due to chronic HBV infection (Sarin et al 1996). Moreover eighty percent of hepatocellular carcinoma cases in India are due to HBV (Sarin et al 1998). The incidence of acute hepatitis B among Indian population with sporadic hepatitis varies from 19.8% to 34.5% and overall, the adult population demonstrates HBV as an etiological agent in 42% of cases of acute hepatitis, 45% of cases of sub-acute hepatitis and 33% cases of fulminant hepatitis (Tandon et al 1984, 1996).

The transmission of HBV infection in the Indian families could be dependent on multiple factors. Very limited data is available on transmission pattern among the family contacts of patients with acute
hepatitis B infection.

In an Indian study, family members of 67 acute hepatitis patients were studied. HBsAg positivity in the spouses was 11.3%, which is significantly higher than that seen in the general healthy population (3.8%). 16% children and 18% spouses became HBsAg +ve in the observation period of 4 months, 19% of spouses were found to be asymptomatic but HBsAg+ve; after 7 months (Dhorje et al 1985). Chronic HBsAg positive subjects are most neglected as far as medical attention is concerned, since majority of these subjects remain asymptomatic for years. So far several mutant forms of HBV have been described by different investigators (Valliammai et al 1995, Kumar et al 1996) Single or multiple point mutations in the pre-core region at codon 28 or 29 preventing HBeAg secretion have been reported. It has been also shown that clustering of mutations in core region is often associated with precore mutations.

The frequency of involvement of the family members of chronic HBV infected subjects and liver disease patients has been found to be very high in India (Dhorje et al 1985). This is almost similar to what has been reported from Africa and China.

The information available on hepatocellular carcinoma (HCC) from India is very limited. It have been found that p53 gene mutation is not a common feature of HCC in India (Katiyar et al 2000). On the other hand, serological profile of HCC has shown that nearly 43% of all HBV related HCC in India are due to HBeAg minus and due to HBsAg undetectable form of infection (Sarin et al 1998).

It remains to be seen whether the infection caused by these patients with HCC to their family members is of a similar viral type or different. This is of great importance since the detection of HBsAg negative group would only be possible by doing HBVDNA / IgG antiHBc studies (Saxena et al 1998, Saraswat et al 1996). In a randomized controlled study, prevalence of the two main HBV mutants was determined serologically. One hundred and twenty patients with chronic liver disease, who were serologically and histologically characterized, were followed up for almost five years (Guptan et al 1998). Over one
fourth (25.8%) of these patients (31 out of 120) were found to be infected with either precore or surface mutant form of the virus. Precore mutations were suspected in 18 (15%) and surface mutations in the remaining. Precore and surface mutation were confirmed by direct sequence analysis in 5 and 7 patients respectively.

The clinical profile of patients infected with precore, surface and wild type HBV were compared. Patients infected with mutant forms were younger than the wild type, though the differences were not significant. Patients with precore mutations were always symptomatic and had a short duration of illness. These patients also showed a higher alkaline phosphatase levels. On the other hand, subjects with surface mutant infection more often had quiescent cirrhosis or hepatocellular carcinoma at presentation.

However, there is great scarcity of data from India on mutant forms of HBV. Their elaborate molecular characterization, prevalence, transmission and significance need to be urgently studied.

2.11 ALT as a surrogate marker for HBV infection

The measurement of liver secreted transaminases specially alanine amino transferase (ALT) started way back in the 1950's, but lost its popularity to detect HBV hepatitis once Australia antigen was detected (Prince 1968). The present relevance of raised ALT in a case of HBV infection has much importance in denoting the host immune response against HBV, since an effective CTL response against HBV is mostly manifested as rise of ALT in the serum. Studies have demonstrated that the loss of HBV, whether spontaneous or interferon induced, is preceded by a state of flair in hepatitis and rise of ALT in the serum. This event is followed by loss of HBV DNA / HBeAg and development of HBeAb. This phenomenon is known as seroconversion. The normalization of serum ALT levels is taken as surrogate marker of inhibition of hepatic necro inflammatory process. Thus serum ALT in case of hepatitis due to HBV is also an important indicator of denoting.
the extent of liver inflammation, though histological grading of the hepatitis is the ideal way to know the extent of viral pathology (Wroblewosky 1959).

2.12 Histological activity index (HAI)

Histological activity index reflects the basic necro inflammatory activity in the liver. In HBV related chronic hepatitis HAI can predict the severity and possibly the progression of liver disease (Knodell et al 1981). HAI is a numeric score which can also be used to predict therapeutic response. It is also the only way to assess progression of disease in asymptomatic chronic HBV infected population who otherwise appears healthy on clinical examination (Ishak et al).