6.1 Profile of Index patients

India comes under the intermediate zone with HBsAg prevalence of 2-7% in the general population. Close family contacts of HBV infected subjects constitute the high-risk group due to multiple modes of transmission. The risk of transmission depends upon: (i) the host (ii) the infectivity of the host, and (iii) the nature and duration of exposure of the family contact to the most. There could be three common clinical situations in which HBV could be transmitted to the contact, namely acute HBV infection, chronic HBV infection and chronic liver disease.


India has the second largest pool of HBV infection in the world and therefore intrafamilial transmission in families of chronic HBV infected patients has great significance specially when all markers of HBV infection and molecular techniques like polymerase chain reaction are used. These techniques can not only detect HBV transmission but also diagnose HBV genomic mutations which are known to have adverse outcome to the natural course of HBV infection. Most of the earlier studies have not utilized these techniques.

In this large study from the Indian subcontinent, 154 liver biopsy proven HBV related chronic liver disease (CLD) patients and 749 family contacts were included. Fifty healthy controls and their 210 family contacts were included as controls. Probably this is the largest series of
biopsy proven HBV related liver disease patients. In a Spanish study by Porres et al (1989), 285 index patients were included but liver biopsy was not done in any. Similarly in an Asian study, 115 index patients were included and inclusion criteria was mere HBsAg positivity (Toukan et al 1990).

The mean age of the indexed CLD patients enrolled in the present study was 35±12 years. All of them had biopsy proven chronic liver disease, which takes at least 2-3 years to develop. The exposure of the contacts to the infected index patient was more than 2-3 years which may provide ample opportunities for transmission of HBV. It is also important to know that none of our index cases had a history of IV drug abuse or high-risk behavior, a common finding in the Western patients with HBV infection. Index patients enrolled in the present study showed a male predominance, (males more than 4 times greater than the females). An obvious genetic determinant of disease outcome is the sex of the host (London et al 1977). Males are approximately 1.5 times more likely to develop chronic HBV infection than females. One contributing factor is the slower plasma disappearance rate for HBsAg in males compared to females (Craxi et al 1982). This observation further confirms male predominance of chronic HBV infection as reported in the Europeans, Africans, Americans and the Chinese (Vegnente et al 1992, Porres et al 1989, Karim et al 1991, Elia Kim et al 1978, Lok et al 1987). In these studies, index male to female ratio was 38:22, 19:11, 197:88, 9:2 and 142:98 respectively. It also possibly supports the preliminary findings of Blumberg that chronic HBV state segregates in a pattern consistent with simple autosomal inheritance disorders (Blumberg et al 1972). It also supports the current concept of HLA mediated male predominance in HBV infection (Thursz et al 1995).

An interesting finding of this study had been that a majority of our index patients came from middle or low socio-economic status. This observation is consistent with data coming from other developing countries (Chen et al 2000, Toukan et al 1990, Szmuness et al 1978). The economic basis of HBV has been demonstrated by only a few studies in India, mainly slum dwellers, tribal, etc. In fact, some authors call hepatitis B a social disease (Jain et al 1992, Murhekar et al 2000).
There are no clear explanations for this linkage of poverty and HBV disease (Chen et al 2000), but it could be linked to poor hygiene, low level of social awareness, high parenteral risk specially in early life. Sub Saharan African where one of the poorest inhabitants of the world live, studies have demonstrated that there is continuous non-uniform acquisition of HBV with advancing age, predominantly through horizontal transmission in early life. A study from Ghana, South Africa have demonstrated that household remains the primary place of HBV transmission with significant association to sharing of bath towels, chewing gum or partially eaten candies and tooth brushes (Martinson et al 1998). The fact that large number of siblings were found to be affected in the present study, could be explained by their close contact and repeated exposure to the infectious source.

Majority (77%) of the index patients in the present study did not give a history of a known contact of HBV infection or were aware of the epidemiology of HBV. Only 22% patients could give a definite history of a known HBV infected subject in the family or were aware of such a possibility. Thus, there was a large gap in the awareness on the such intrafamilial spread of HBV was observed. Most of the patients learned about the disease transmission, or the importance of family screening only during the course of this study. This lack of information, education and communication in our group of HBV related CLD patients is similar to the findings of low awareness seen in other blood born infections in developing countries where proper dissemination of information on the blood infections is not available in mass scale, specially for similar diseases like AIDS (NACO) India, 1994). This low level of awareness about this disease is the biggest limitation in controlling the transmission of HBV in developing countries.

Only 37% of the index patients had a positive history of parenteral risk to HBV infection; 24% of them gave a history of blood transfusion while 13% had an operation or intervention. Parenteral risk for HBV is of high importance in India where safe blood banking practices are not adequately available. In a recent Indian study (Saxena et al 1999), 818
of blood was transfused after screening of HBsAg using conventional enzyme immuno assay. The recipients were followed up for the risk of transfusion-associated hepatitis (TAH). In a mean follow up period of six months almost 14 (7.6%) recipients developed hepatitis. of these three (21%) were TAH-B. The respective donors of these three recipients were tested for HBsAg using polyclonal EIA, they were found to be HBsAg positive. Irrespective of the anti-HBc status, about 9% of the healthy adults in this study who are HBsAg negative by EIA are HBV DNA positive. In such persons, the HBsAg negativity could be due to the low sensitivity of the assay system used, low level of antigenemia, or the existence of surface mutant strains which are missed by the conventional EIA.

In majority of the index patients the mode of acquisition of HBV was not clear. This high number of patients presenting with sporadic HBV opens up a large debate on the possible modes on HBV transmission in Indian population.

6.2 Prevalence of HBV infection in contacts:

The results of the investigations in 749 family contacts of this series of 154 index CLD patients have revealed many important findings on the epidemiology of HBV infection in the Indian households. In 75% of families (115 /154), one or more family member was found to have at least one marker of hepatitis B virus infection or exposure (positive families). This prevalence was significantly higher than the positivity seen in the contacts of control population (p<0.05). Prevalence of HBsAg positivity in this group of patients was high. Of the 749 family contacts studied 18.3% were HBsAg positive and 28% of them had isolated IgG anti-HBc positivity. HBV DNA was found to be positive in 49% of HBsAg positive people and 61% of isolated IgG anti-HBc positive cases. This probably is the largest series studied in the world where such large numbers of close family contacts were subjected to HBV detection by molecular techniques to unravel the potentially infectious reservoir of HBV was used. The observation of a high prevalence of HBsAg positivity within the families of chronic HBV infected patients in India opens up
serious concern to evolve new strategies to curtail this infection in our
country.

6.2.1 Prevalence of HBV infection in family contacts: Meta analysis of
Indian studies

Even though India constitutes the second largest pool of chronic HBV
infected people in the world, there is paucity of data on the intrafamilial
prevalence of HBV. Till date, a total of 5 studies besides the present one.
have been reported. The other 5 studies have appeared only in abstract
form and hence only limited information could be gathered (Table29).
Chakraborty et al. studied 264 contacts of 90 index chronic HBV cases.
HBsAg positivity was seen in 23%. In the other four studies on an
average 100 family contacts were studied. The major limitation of these
studies beside a small sample size and the lack of information on
markers of HBV exposure, IgG anti-HBc, HBeAb and marker of infectivity
HBV DNA. Also importantly, none of these studies included a control
group and hence useful conclusions can not be arrived at.

The present study of 154 index patients and 749 contacts
showed not only a high HBsAg prevalence (18%) in family contacts but
also demonstrated that in addition 28% of family contacts have markers
of past exposure. The fact that 65% of family contacts were infectious
(HBV DNA positive). Sixty nine of the 140 HBsAg positive contacts were
HBVDNA positive while 128 of 210 IgG Anti-HBc positive subjects were
HBV DNA positive. Over all 69% of the family contacts had circulating
HBV DNA. An analysis of all these Indian studies reveals that of the total
available 256 indexed HBV cases and 1437 living contacts studied,
HBsAg prevalence was found in the range of 6-45% with a mean of 21%
(Table 29). This is comparable to 20% HBsAg prevalence reported
from rest of the world (Table 31). In the world literature there are only a
few large studies. In a study from Australia by Macintosh et al (1998).
and another from Germany by Santantonio et al (1997), HBV DNA
positivity was found in 20-51% of HBsAg positive family contacts. These
figures are lower than the HBV DNA positivity rate seen in the Indian
population. Notably in this study probably for the first time in the world
investigators could demonstrate that almost 61% of HBsAg negative family contacts are also HBV DNA positive. This opens up a new area for research in these exposed contacts. We don’t know the significance of this high HBV DNA positivity rate in HBsAg negative contacts.

HBsAg prevalence in close family contacts from other parts of Asia ranges from 10-30%, with mean value of 17% (Table 30). The positivity of past exposure ranged from 26-55% with a mean value of 42%. These prevalence rates are comparable to data of present study. Globally documented data reveals HBsAg positivity in the range of 6-66% (mean 29%). This figure is much higher than the finding of the present study. Most of the studies reported from USA, Australia, New Zealand etc included immigrant families from South East Asia which is a hyper endemic zone for HBV. Prevalence of past exposure in these studies ranged from 27% in Spain to almost 70% in Western Africa. None of the studies from India or Asia had involved detection of HBV DNA. However, two studies from Germany and Australia have done HBV DNA detection but that was only in HBsAg positive subjects. Transmission of HBV infection within family also depends upon the number of infected subjects present in the family. The relative risk increases with the increasing number of infected subjects.

In a study reported from Jordan, (Toukan et al 1990) a high endemic zone of HBV, out of the almost 1,115 family members studied only 9.9% had evidence of HBsAg positive infection. In the present study, there was also a sequential increase in age related HBsAg positivity within families. At 0-10 years the HBsAg positivity rate was 19%, which increases to 21% at 20 years and attained a peak at 41-50 years to 25% followed by a declining trend. There is no clear cut explanation to this comparatively low HBsAg carrier state within the families of our study but it is very much possible that most of the HBsAg positivity acquired through house hold contacts came later in life. It is also probable that families had a high percentage of immigration rates of their young population due to economic reasons. Thus we could have missed a large majority of positive people. Studies in China and developed countries have shown that HBV attains 80-90% chronicity only when the virus is

6.3 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. The exact pathogenesis of familial aggregation of HBsAg remains unknown. Clustering of HBV infection was found in 42% of the families studied. The clustering phenomenon seen in families of CLD patients was significantly higher than the families of healthy controls. Number of affected members included two in 25%, 3-4 in 12% and more than 4 members in 10% of the families studied. This high incidence of family clustering is comparable to the reports coming from China and other Asian countries. East and South East Asia are among the most populous regions in the world and are made up of countries with different degrees of prosperity and in different stages of industrialization. Hepatitis B infection is hyper endemic in this region. In a study from Israel (Bisharat et al 1998) seventy eight families including 506 members were investigated. HBsAg positivity was 16%. In 40 families (51.3%) at least one family member was HBsAg positive and in 19 families (24.4%) two or more members were HBsAg positive. China has one of the highest rates of hepatitis B endemicity in the world. In a survey of five Chinese provinces, the overall HBV infection rate in the general population was found to be 42.6% with 10.3% HBsAg positivity. In a nation wide survey, 27.2% of families were found to have one or more HBsAg positive members revealed a strong tendency of family clustering (Yao et al 1996). In Korea it has been shown that the household transmission of HBV, especially from parents to offspring resulted in intra familial clustering and was almost 6.6 times higher than that of the controls (Young et al 1993) In Jordan, an endemic zone of HBV, out of the almost 1,115 family members studied only 9.9% had evidence of HBsAg positive infection. The frequency of exposure to HBV was 36%, the infection rate increased from 57%
of a single carrier family to 98% when there were 3 carriers in a family. HBsAg prevalence was zero at 1\textsuperscript{st} year, 11% at 2\textsuperscript{nd} years, 90% at 3\textsuperscript{rd} year (Toukan et al 1990). Family size plays an important role in clustering phenomenon. In the present study 60% of the families were of joint type where more than one generation and more than one related families were living together. Moreover in 60% of the families 5-6 or more than six members were living together. Thus familial clustering was a common phenomenon in family contacts. It is possible that repeated exposure due to close proximity to infected individuals within families probably contributed in the clustering process.

Majority of the contacts in the present study were asymptomatic and were found to be infected only during family screening. Detection of HBV DNA revealed presence of circulating DNA even in 61% of the contacts having just markers of past HBV exposure. This group of individuals often otherwise are considered to be having resolved infection by the conventional epidemiological understanding. This large group of asymptomatic population carrying such high prevalence of HBV DNA within families probably contributes as a potential pool of infection, and generates a clustering process.

6.4 Routes Of HBV Transmission:

The routes of transmission of HBV differ in various parts of the world. The predominant routes of transmission of HBV vary according to the endemicity of HBV infection. In areas of high endemicity, perinatal transmission is the main route of transmission, whereas in areas of low endemicity, sexual contact is the predominant route.

Studies have demonstrated that infected virions could be present in urine, bile, tears, sweat, breast milk, saliva, semen, menstrual blood of infected patients (Martinson et al 1998, Chen et al 2000, Zhevachevsky et al 1999, Davidson et al 1987). Thus the household contacts are at high risk for HBV transmission from vertical, horizontal, sexual, vector born and possibly unknown modes of transmission (Chen et al 2000, Maddrey et al 2000). The HBV transmission within families is not only acquired by multiple routes but is
also influenced by prevalence of HBV within general population. In regions of high endemicity, transmission studies have demonstrated high mother to infant (Perinatal mode) transmission (Maynard 1990, Schweitzer 1975, Alter 1996). In fact these regions have a lifetime risk of HBV acquisition greater than 60%.

United States which has one of the lowest endemicity for HBV, the most common modes of transmission is through unsafe sexual activity and intravenous drug abuse (Hollinger et al 1990) . where as Perinatal transmission is common in South East Asia.

In the present study, probably horizontal mode is the major mode of transmission. The most important finding of this study had been the high prevalence of HBV infection in first degree relatives of our index patients. The HBsAg positivity rate in them was almost double the prevalence in 2° or sexual contacts. The siblings in the family were the most affected and were independent of the HBsAg positive status of mother, as out of the 154 index cases in this study only 29 (19%) were mothers. More over only 6% of the contacts gave a history of parenteral exposure. This observation suggest that majority of contacts have acquired HBV infection through family contacts. These observations further support the horizontal mode of spread of HBV in families included in the study.

Research on the routes of spread on HBV within population has opened up many debates on the epidemiology of HBV within populations. An analysis of important studies from world literature reveals that the primary mode of transmission within families is horizontal irrespective of the endemicity of HBV in that area (Table 32). In the present study it has been observed that transmission of HBV was horizontal, vertical and sexual in 71%, 15% and 8% contacts respectively. The finding of high horizontal spread among family contacts is similar to pattern observed in other part of the world (Table 32).

Thus the findings of this study are very important from the public health point of view so as to develop effective and economical vaccination strategies. In most of the South East Asian countries especially in China, Taiwan etc. the endemicity rate in general population
is almost 60-90%. Here, easy mother to child transmission of virus has posed many problems to the vaccination strategies, as vaccine escape phenomenon is commonly seen in their population. Indian population seems to acquire HBV only during early childhood i.e. more than two years of age mostly by horizontal mode (Thyagrajan et al. 1996). This was very well confirmed in this longitudinal study, as most of the children in study acquired HBV infection after 2 years of life. The reason for predominant horizontal mode of transmission seen in the contacts of the present study could be primarily due to (a) low incidence of HBsAg positivity rate in pregnant females (Maheswari et al. 1985, Sehgal et al. 1983) (b) low incidence of HBsAg positivity rate in general population and (c) high parenteral exposure in early childhood.

A sero-epidemiological study was conducted in an orphanage among children of 5-16 years. The HBsAg carriage was 15.3% while 56.5% were positive for anti HBs (Sehgal et al. 1983). The overall exposure to HBV infection was 71.8% as compared to 8% of school going population. The prevalence was higher among boys living there for more than 3 years as compared to those with a shorter stay. Thus duration and frequency of exposure are the key factors for the risk of HBV infection.

In another study 193 household contact of 40 HBsAg positive hepatitis patients and 103 contacts of 27 HBsAg negative hepatitis patients were screened for the presence of markers of HBV (Dhorje et al. 1985). The family contacts of HBsAg positive patients had a significantly higher prevalence of HBV infection; HBsAg was 11.34% in the first group while 3.8% in the second group. Similarly the anti HBs was 25.3% in the contacts of HBsAg positive hepatitis patients while it was just 13.5% in HBsAg negative subjects. However, the absence of anti HBe IgM in the HBsAg positive contacts indicates that they had most probably acquired the infection prior to the patients. Though the index subject in this study had acute viral hepatitis B, still conveys the message that horizontal transmission is the primary mode within the families. These two studies clearly demonstrate the various dimensions of horizontal transmission of HBV infection in Indian settings.
6.5 Spectrum of histopathological features of HBV mutant infections in family contacts:

Of the 140 HBsAg positive contacts, 69 (49%) had detectable HBV DNA in the serum. Of these, 32% were HBeAg and HBV DNA positive and 17% were HBeAg negative, HBeAb positive, HBV DNA positive. We could not study liver biopsies except in one of the HBsAg negative, IgG anti-HBc positive contact due to lack of consent and medical indication. The significant findings of our investigation had been, high incidence of chronic active hepatitis. Almost two third of our family contacts irrespective of their serological status (with or without HBeAg) had evidence of chronic liver disease. Importantly most significant finding had been the presence of high incidence of HCC in HBeAg negative, HBeAb positive family contacts. Almost 19% of them had HCC, which was significantly higher (p < 0.05) than the prevalence seen in HBeAg positive, wild type infected contacts during the same period. The sequencing revealed that majority of the mutant infected contacts demonstrated point mutations in the 1896 or 1898 G to A mutation. The S gene was conserved in these individuals. The high probability of mutant population developing severe disease and HCC is similar to our findings earlier in Indian population (Gupta 1996). Zong et al (2000) recently has shown that the presence of Pre C mutant involving codon 28 and 23 in the tumor tissue.

HBeAg present in excess in the serum of HBsAg carriers is indicative of high replication and infectivity. Seroconversion to HBeAb is generally accompanied by rapid decrease in viral load and possibly subsequent and indolent course for many years, finally loosing the HBV infection. Studies have demonstrated that this loss of HBeAg from the serum in a proportion of cases can be due to the presence of TAG stop codon mutation in the pre core region of the HBeAg gene (Lindh et al 1995). A selection of this nucleotide 1896 G→A mutation may occur in a highly immuno active stage of HBV infection. Occurrence of TAG mutation is known to be associated with fulminant form of hepatitis in family contacts (Liang et al 1994) and severe chronic hepatitis (Brunetto...
et al. 1989). There was no clear explanation for high incidence of HCC in pre-core mutant infected individuals. The only possible hypothesis was that these group of patients develop a cirrhosis of the liver much frequently and earlier due to the high severity of hepatic activity, which progressed into liver cancer. Recently, the molecular mechanism of this classical HBeAg negative, HBeAb positive, pre-core mutant infection developing liver cancer has been conclusively explained.

The study by Kramvis et al. in 1998 has suggested that pre-core mutants disrupt viral replication process but it promotes viral genomic integration of uncapsidated replication intermediate resulting in hepatocellular carcinogenesis (Kramvis et al. 1998).

The only HBsAg negative DNA positive family contact histologically examined in this study revealed a series of mutations in the conserved "a" determinant of the S gene as well as the flanking regions. The individual concerned was a female 35 years old, married, with three children. Her father also had similar mutations, who was the index patient and had died of advanced liver disease and HCC earlier. A number of mutations, including amino acid 137, 138, 145 of the "a" determinant were observed. Histopathologically she demonstrated chronic hepatitis but a relatively milder disease. She was asymptomatic and was leading a normal life. It is very difficult to comment on the pathological significance of HBsAg negative mutants of the S gene in the family contacts. But earlier studies have demonstrated a high incidence of viral integration and liver cancer in HBsAg negative infection. Incidentally the lady concerned though had HBsAg negative profile, her father who died of liver cancer had a complete wild type serology. Thus it is very much possible that there might be a mixed population of wild type and mutant viruses circulating within this family. Thus it will not be worthy to comment on the carcinogenic potential of this form of mutation.

6.6 Prevalence of Probable HBV mutants in family contacts:
Serological analysis

In last 50 years of research in the epidemiology of HBV infection, starting from the first description of Australian antigen by Blumberg et al.,
many studies have reported HBsAg or anti-HBS prevalence in family contacts of HBV carriers but very few studies have attempted to study the prevalence of HBV DNA positivity and existence of HBV mutants in the families (Beasley et al 1997, McIntosh et al 1998, Wallace et al 1994). In this large series of family contacts studied, detailed molecular diagnosis was used for the first time to detect the prevalence of HBV within families. Of the 140 HBsAg positive contacts detected in the study 32% were found to be HBeAg positive, 31% had HBeAb and rest 37% had isolated HBsAg. HBV DNA positivity in the HBeAg positive subjects was 100% (probable wild type infection), while 55% of the HBeAg negative/HBeAb positive subjects were DNA positive (probable 'precore mutant'). In the HBsAg negative contacts, 21% were HBV DNA positive. We could detect HBV DNA in two consecutive samples with PCR. It is well known fact that HBV DNA PCR analysis is a function of (a) PCR conditions (b) Primer selection. Since for the detection of HBV genome (diagnostic) we have not used nested PCR, it is possible that some of the HBsAg positive/ HBeAg negative/ HBeAb positive family contacts who had low level viremia, could have been missed by our HBV DNA determination method. It is well known that these low viremic HBsAg positive subjects can transmit HBV infection, if their blood is transfused to healthy people. Thus the infectious potential of this pool of low viremic, possible mutant HBV population cannot be ruled out in the families studied (Weizsacker et al 1995). This serological pattern of HBsAg positive contacts is in conformity with the data from Middle East (Toukan et al 1990) where HBeAg and HBeAb was present in 32 and 39% of HBsAg positive family contacts, rest 29% had isolated HBsAg. Earlier studies have also documented the presence of precore mutants in the family contacts (Hannoun et al 2000, Santantonio et al 1997). Akarca et al (1994) have found the presence of precore mutation in 33% asymptomatic HBeAb positive family contacts.

An important observation of this study had been isolation of HBV DNA from the serum of many isolated IgG anti HBC positive HBsAg negative family contacts (possible 'surface' mutants). Over all, there was a tendency for increased mutant profile in our families. The history of
HBV surface mutants dates back to the 1980. A group of 44 children in Italy who had received the hepatitis B vaccine and HB Ig were followed up between 1982-1987. Thirty two of them had protective levels of anti-HBs and also the markers of HBV replication. The subjects with IgG anti-HBc as the only marker of HBV infection were studied in a Canadian study by Scully et al. (1994). Thirty six HBsAg negative, anti-HBc positive patients were investigated and 7(19%) were found to have ongoing continuing HBV replication and hepatic inflammation. In a recent study (Weinberger et al 2000) serum of 357 subjects with IgG anti-HBc as the only marker of HBV infection, were tested for HBV DNA. Thirty three (9.2%) were found to be positive by nested PCR with a generally low virus load (mean 5 × 10^7 per ml). The HBV DNA positivity in anti-HBc positive subjects is thus known to vary in different geographical region of the world. In such persons HBsAg negativity could be due to the low sensitivity of the assay system used, low level of antigenemia or the existence of surface mutant strains in the population.

6.7 Types of mutants in family contacts: Molecular Analysis

In the present series of 749 family contacts studied, three serotypes of HBV infection in the family contacts were isolated. One hundred and forty (18.3%) contacts were HBsAg positive. In the 609(82%) HBsAg negative group, 28% were IgG anti-HBc positive. Further analysis of the positive contacts revealed 3 serological patterns. In the HBsAg positive group (group a) 32% were HBsAg positive/ HBeAg positive/ DNA positive (wild type) (group b) 17% were HBsAg positive/ HBeAg negative/ HBeAb positive/ HBV DNA positive (possible Precore mutant), and in the isolated IgG anti-HBc group (group c) 21% were HBsAg negative/ IgG anti HBC positive/ DNA positive (possible Surface mutant). It was interesting to know that the pattern of HBV spread within families followed a serological pattern. Very high incidence of possible mutation within family contacts was characterized serologically.

Most HBV genomes of known infectivity express five proteins: Envelope protein (HBsAg) encoded by S-ORF, nucleocapsid
protein (HBeAg) encoded by C-gene, the e antigen (HBeAg) encoded by the Pre-C/C gene, the polymerase encoded by the P-gene and HBx proteins encoded by the X-gene. In a chronic HBsAg positive subject however, substantial heterogeneity of HBV genomes which can interfere with the expression of any of these genes has been demonstrated (Thomas 1995, Blum 1993). HBV variants with mutation in the Pre-C region unable to express HBeAg have been reported commonly in the general population and families of chronic HBV infected patients. Precore mutant is known to be easily transmitted sexually within the families and causes fulminant hepatic failure, severe liver disease, propensity for HCC, increased fibrosing cholestatic hepatitis. In addition, few studies have demonstrated dominant populations which can not express pre S protein. Experimental studies have also demonstrated that the major S protein mutations resulting in HBsAg-ve/ HBV infection are also highly infectious (Wallace and Carman 1994). However, it is still an open question which of the various variants of HBV are transmitted within families and persist as stable strains. Presently, only a few studies have demonstrated the possibility of intrafamilial transmission and clustering of mutant HBV as demonstrated by sequence analysis of HBV genome.

There are several methods by which a mutant can be detected. These are namely (a) single strand conformational polymorphism: It is based on the principle that presence of mutation changes the mobility of the amplified fragment observed in the electrophoresis. The limitation of this method is that it does not detects the mutation specifically. (b) Mutation specific PCR: The primers for the PCR are designed with change in nucleotide according to the known mutation. In absence of a mutation in the specimen amplification does not take place. (c) Sequencing: Direct sequencing of PCR product can be done by using chain terminator reaction with radioactivity or fluorescence dye. Of the above-mentioned methods direct sequencing is the gold standard as there is negligible chance of artifact.

In a Chinese study, patients of asymptomatic HBsAg chronic infection were studied (Akarca et al 1994). 53 index cases and
89 of their close family contacts were studied with the objectives: a) prevalence of precore mutation in asymptomatic carrier and b) whether family members share the same mutant sequence as the index patient. Direct sequencing of PCR amplified products of Pre-C region demonstrated a stable mutation Mo (T to C at nucleotide 1858, at codon 15). In 81% of the family members who carried similar mutations, clustering of M0 mutation was also demonstrated, however M1 mutations was found in quite few instances (C to T change at nucleotide 1856 proline to serine, codon 15) in respective family members and index cases. Many other mutations in precore gene were also detected in index as well as contacts but with relatively lower homology or reproducibility (Beasly et al 1997).

In this series of 749 family contacts it was not possible to sequence all the family contacts and the index cases. However of the 154 index chronic liver disease patients, eleven index subjects along with every affected contact were randomly selected for the sequencing. This included a total of 26 cases, who were sequenced for pre-core and S-gene. This also included a complete family of HBeAg +ve end-stage liver disease cancer patient, along with the third degree contact, a servant was also sequenced. The serological pattern of these sequenced patients was, Six HBsAg +ve / HBeAg +ve/ DNA +ve (Wild Type) and five HBsAg +ve/HBeAg -ve / HBeAb +ve / DNA +ve (Precore mutant) index cases with their respective contacts.

Molecular characterization of pre-core and S-gene within families could reveal definite evidence of mutations in the suspected serologically characterized patients. The precore gene analysis revealed common mutations at nucleotide 1896 and 1898. It is interesting to note that there was ~ 100% sequence homology between all the index cases and their contacts. This supports the intrafamilial transmission of HBV infection and familial clustering of HBV mutations. All the sequenced index cases and their contacts had HBeAg negative serology. This support the hypothesis of horizontal transmission and spread of HBeAg negative HBV to the contacts. In the 6 wild type index cases and their contacts, expectedly no mutation were present in pre C gene but incidentally on sequencing S gene many novel mutations were found.
which were not reported earlier in family contacts. These mutations were in the upstream flanking region of the major "a" determinant starting from amino acid 124 to 147. The commonest mutations found were at amino acid 118 and 128. Besides these other mutations seen were at AA 114,117, 120, 124, 126 ,127 ,129,132,135,137,138, 142,143 and 145. These observations in serologically distinct HBsAg positive individuals clearly indicate that HBV infection exists as a quasispecies and mixed infection of wild type and mutant viruses. These patients were non-treated and unvaccinated, and hence, the various viral mutants seen had emerged spontaneously over the course of time. The exact significance of this mixed viral population is not clear. There are very few instances of direct observations made on such clinical phenomenon. Lok et al. have demonstrated in Chinese patients that family contacts with mixed wild type and pre Core mutant infection can have a altered course in natural infection. They have demonstrated that only 46% of HBeAg negative patients with mutation in Pre C gene have active liver disease, while almost 95% of the of patients with HBeAg positive infection and mixed Pre C mutations have a severe disease. This severity in liver disease in such mixed viral populations is unclear and need more research to achieve conclusive opinion.

Another interesting finding of the sequence analysis was that in spite of the Glycine to Arginine change at AA 145 there was no loss of HBsAg . In another subject who was HBsAg negative and had a G145R mutation, a change from Cystine to stop codon was found at amino acid position 137 and 138. These two are the novel mutations and were not reported earlier. The pathological significance of such S mutations is not clear. It has been earlier demonstrated that mutations in the flanking regions of the “a” determinant of the S gene can result in destabilization of the pre-genomic RNA and can result in HBsAg negative status ( Banerjee et al 1999 ). Few studies have also demonstrated that mutations in the S gene at amino acid 145 can result in HBsAg negative status, especially when conventional monoclonal EIA are used (Carman 1991). The mutation reported in these two studies were at amino acid 118 and 145. In the present study these mutations were found in the patients with HBsAg positive serology. This observation suggests that HBsAg negativity is not necessarily due to the presence of these
mutation in the "s" gene. However cloning and expressing the gene products in cell culture systems can help in demonstrating the significance of these mutations. Viral expression systems can not only study gene expression of mutated virus but also can help to test the replicative competence of these strains under influence of antiviral agents.

Emergence of mutant forms of hepatitis B virus is a slow process within the families. Under repeated viral exposure, the host immune response puts constant pressure on the acquired virus. During this course of viral infection selective mutations take place in the viral genome, which helps in its survival within the host. Pre core mutants are the earliest and the most common to emerge during this selection process (Carman et al 1995). It has been evident for some time that under normal immunological conditions HBV is non-cytopathic to the host (Chu et al 1985). Hepatitis results from immune recognition of the virus infected cells by the host. Cytotoxic T-lymphocytes (CD8 +ve) are especially directed towards virus encoded proteins and assess the immune pressure/recognition process (Penna et al 1991). The recognition of viral peptides derived from any of the virus encoded protein, surface, core, polymerase, and the X protein is under mediation of MHC class I or Class II type cells. These processes are also under the influence of a variety of cytokines including alpha / gamma interferon, interleukins, etc. The action of the CD8 mediated cytotoxicity is followed by immune response from the humoral arm of the host immune system resulting in HBV clearance.

If this process of CD8 induction followed by humoral antibody development towards the respective viral antigens is ineffective, than viremia can persist. Viremia continues for many years. Initially there is very little inflammatory process in the liver.

As years go by, the level of hepatitis increases presumably due to recruitment of new components of the immune system and importantly because of antigenic variation of viral proteins caused by spontaneous mutation of the viral genome due to transcription errors introduced by viral reverse transcriptase. The
immune pressure during this period of antigenic variation is primarily CD4 mediated. The role of CD4+ve lymphocytes in this process is supported by studies which have demonstrated sequential increase in CD4 activity during HBeAg/anti-HBe seroconversion, when possibly HBeAg –ve mutants can be selected (Tsai et al 1992). This CD4 activity presumably mediates the action of B lymphocytes which produce increasing quantities of immuno-globulins to bind HBe and HBc proteins. It is also suggested that some components of the immune system may be responsible for the control of the virus during latent infections, immuno-suppression or patients with certain phenotypes which present viral peptides with low affinity and induce poor cellular responses. It is also likely that the re-emergence of virus with mutant strains is accompanied by presence of anti-HBe and inability to produce HBeAg due to a selection pressure on the virus. Studies have demonstrated a conversion of codon 28 of the pre C gene to a translated stop codon resulting in loss of HBeAg production. This concept of a new virus species emerging due to mutations in the antigenic epitopes is further supported by the recent observations of emergence of immune escape phenomenon caused by the rapid mutation in the core gene. The intriguing aspect of this viral mutation had been the possibility of vigorous immune response when the mutant virus is transferred to a new host like a family contact. Several groups have reported high incidence of fulminant hepatitis in sexual contacts of pre core mutant HBV index patients (Carman et al 1991) and acute sever hepatitis in a case of HBsAg negative blood transfusion (Carman et al 1995).

Molecular analysis of S gene in index and contacts of 11 families has shown the presence of identical mutations in index and its contacts. A total of 33 DNA's were sequenced which included 25 HBsAg positive, and six HBsAg negative subjects in the study group. In the control group there were only two specimen which were HBsAg negative /IgG anti-HBc positive/HBV DNA positive.

In the HBsAg positive group of the 25 sequences 21 (84 %) and in the HBsAg negative group of the six only one (16%) had mutation in the "s" gene. Sequences of the control group were wild type. Same specimen were sequenced for the precore region. This included fifteen
HBeAg positive/HBV DNA positive, 10 HBeAg negative / HBeAb positive/DNA positive and six IgG anti HBc /HBV DNA positive subjects in the study group. Control group included two IgG anti HBc /HBV DNA positive subjects. All (100%) the HBeAg negative/ HBeAb positive subjects showed presence of mutation in the precore gene. However none of the HBeAg positive subjects had mutation. The sequences of the S and pre C region in study were compared with the consensus HBV sequence of the Gene bank and aligned using the online Mult Alin software. The most interesting observation of the sequencing analysis is the presence of G145A mutation even in the HBsAg positive (3/25, 12%) subjects and 118 and 128 were the commonest mutations. Presence of similar mutation in the sequences of the index and its contacts is in favor of intrafamilial transmission of HBV mutant. In more than 70% families the mode of transmission is horizontal and family number 10. It is horizontal as well as vertical. Vertical transmission of S mutant G145R is well known similarly mutant 126, 129 and 133 were also transmitted vertically (Oon et al 1998) recently Oon et al (2000) have demonstrated the horizontal transmission of S mutant G145R. This mutant was identical in family members of 3 of the ten infants. The present study reaffirms the observation of Oon et al. In family number 10 the same mutations G145R was found in even third degree contact, a servant who was living in the same premises with the other affected members with identical mutation in the S gene.

Overall the present study reveals that family contacts of HBV related CLD patients or at very high risk for HBV infection. The family contacts acquire infections by wild type and mutant viruses effectively.
## Table 29: Indian Data on HBV Infection in Families

<table>
<thead>
<tr>
<th>S.No</th>
<th>Area</th>
<th>Year</th>
<th>Author</th>
<th>No of families Studied</th>
<th>No of members studied</th>
<th>HBsAg (%)</th>
<th>IgG aHBc/ aHBs (%)</th>
<th>HBV DNA</th>
<th>Seq.S-gene</th>
<th>Seq.Prec</th>
<th>Diag Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Delhi</td>
<td>2001</td>
<td>Thakur</td>
<td>154</td>
<td>749</td>
<td>140 (18)</td>
<td>28%</td>
<td>50% in sAg +ve &amp; 21% sAg -ve</td>
<td>aa,114,118,120,126,128,131,133,137,138,142,143,145,155</td>
<td>Codon25,28,29</td>
<td>CLD</td>
</tr>
<tr>
<td>2.</td>
<td>Hyderabad</td>
<td>2000</td>
<td>Joshi</td>
<td>21</td>
<td>149</td>
<td>8 (17)</td>
<td>7%</td>
<td></td>
<td>-</td>
<td></td>
<td>Chr. HBV</td>
</tr>
<tr>
<td>3.</td>
<td>Delhi</td>
<td>1998</td>
<td>Bohidar</td>
<td>67</td>
<td>34</td>
<td>2 (6)</td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Calcutta</td>
<td>1998</td>
<td>Chakravarty</td>
<td>90</td>
<td>264</td>
<td>61 (23)</td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
<td>AVH-CLD</td>
</tr>
<tr>
<td>5.</td>
<td>Jaipur</td>
<td>1998</td>
<td>Nijhawan</td>
<td>18</td>
<td>110</td>
<td>50 (45)</td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
<td>HCC</td>
</tr>
<tr>
<td>6.</td>
<td>Chennai</td>
<td>1995</td>
<td>Tyagarajan</td>
<td>50</td>
<td>140</td>
<td>34 (24)</td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
<td>Chr. HBV</td>
</tr>
</tbody>
</table>
Table 30: Asian Data on HBV Infection in Families

<table>
<thead>
<tr>
<th>S.No</th>
<th>Country</th>
<th>Year</th>
<th>Author</th>
<th>No. of families Studied</th>
<th>No. of members studied</th>
<th>HBsAg (%)</th>
<th>IgG aHBe/ aHBs (%)</th>
<th>HBV DNA</th>
<th>Seq:S-gene</th>
<th>Seq:PreC</th>
<th>Diag. Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Israel</td>
<td>1998</td>
<td>Bisharat</td>
<td>78</td>
<td>506</td>
<td>17%</td>
<td>51%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Chr. HBV</td>
</tr>
<tr>
<td>2.</td>
<td>Korea</td>
<td>1993</td>
<td>Kim</td>
<td>51</td>
<td>71</td>
<td>14%</td>
<td>54%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Chr. HBV</td>
</tr>
<tr>
<td>3.</td>
<td>Saudi Arabia</td>
<td>1990</td>
<td>Ramia</td>
<td>10</td>
<td>109</td>
<td>18%</td>
<td>35%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Chr. HBV</td>
</tr>
<tr>
<td>4.</td>
<td>Middle East</td>
<td>1990</td>
<td>Toukan</td>
<td>115</td>
<td></td>
<td>10%</td>
<td>26%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Chr. HBV Jordan</td>
</tr>
<tr>
<td>5.</td>
<td>Honk Kong</td>
<td>1987</td>
<td>Lok</td>
<td>240</td>
<td>731</td>
<td>28%</td>
<td>43%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Chr. HBV</td>
</tr>
</tbody>
</table>
### Table 31: Global Data on HBV Infection in Families

<table>
<thead>
<tr>
<th>S.No</th>
<th>Country</th>
<th>Year</th>
<th>Author</th>
<th>No. of families Studied</th>
<th>No. of members studied</th>
<th>HBsAg (%)</th>
<th>IgG Anti-HBc</th>
<th>HBV DNA</th>
<th>Seq Segene</th>
<th>Seq PreC</th>
<th>Darg Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Australia</td>
<td>1998</td>
<td>McIntosh</td>
<td>34</td>
<td>184</td>
<td>59 (32)</td>
<td>-</td>
<td>20%</td>
<td>aa 120.126, 133.159.165</td>
<td>1896</td>
<td>Chr. HBV</td>
</tr>
<tr>
<td>2.</td>
<td>W. Africa</td>
<td>1998</td>
<td>Francis</td>
<td>121</td>
<td>1385</td>
<td>290 (21)</td>
<td>69%</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>Chr. HBV</td>
</tr>
<tr>
<td>3.</td>
<td>Germany</td>
<td>1997</td>
<td>Santantonio</td>
<td>5</td>
<td>37</td>
<td>19 (51)</td>
<td>43%</td>
<td>51%</td>
<td>Pre S</td>
<td>1896</td>
<td>Chr. HBV</td>
</tr>
<tr>
<td>4.</td>
<td>New-Zealand</td>
<td>1996</td>
<td>Martin</td>
<td>907</td>
<td>2957</td>
<td>710 (24)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Chr. HBV</td>
</tr>
<tr>
<td>5.</td>
<td>Italy</td>
<td>1992</td>
<td>Vegnente</td>
<td>60</td>
<td>249</td>
<td>164 (66)</td>
<td>40%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Chr. Carrier</td>
</tr>
<tr>
<td>6.</td>
<td>Africa</td>
<td>1991</td>
<td>Karim</td>
<td>28</td>
<td>186</td>
<td>37 (20)</td>
<td>54%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Chr. HBV</td>
</tr>
<tr>
<td>7.</td>
<td>Spain</td>
<td>1989</td>
<td>Piores</td>
<td>285</td>
<td>848</td>
<td>51 (6)</td>
<td>26%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Chr. HBV</td>
</tr>
<tr>
<td>8.</td>
<td>U.S.A</td>
<td>1989</td>
<td>Franks</td>
<td>96</td>
<td>1387</td>
<td>124 (11)</td>
<td>37%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 32: Pattern of mode of HBV transmission in families

<table>
<thead>
<tr>
<th>Region</th>
<th>Year</th>
<th>Author</th>
<th>Mode of transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. America</td>
<td>1982</td>
<td>Bernier</td>
<td>Vertical</td>
</tr>
<tr>
<td>2. Hong Kong</td>
<td>1989</td>
<td>Lok</td>
<td>25%</td>
</tr>
<tr>
<td>3. South Africa</td>
<td>1991</td>
<td>Karim</td>
<td>15%</td>
</tr>
<tr>
<td>4. Italy</td>
<td>1991</td>
<td>Vegnente</td>
<td>2%</td>
</tr>
<tr>
<td>5. Korea</td>
<td>1993</td>
<td>Kim</td>
<td>24%</td>
</tr>
<tr>
<td>6. Spain</td>
<td>2000</td>
<td>Porres</td>
<td>41%</td>
</tr>
<tr>
<td>7. India</td>
<td>1992</td>
<td>Jayram</td>
<td>38%</td>
</tr>
<tr>
<td>8. India</td>
<td>2000</td>
<td>Nijhawan</td>
<td>26%</td>
</tr>
<tr>
<td>9. India</td>
<td>2001</td>
<td>Thakur</td>
<td>15%</td>
</tr>
</tbody>
</table>