Hepatitis B virus (HBV) is an important health problem of world wide concern. The frequency of HBV infection in a subset of population depends upon several factors which increase the risk of viral entry in to the body. These, environmental and life style related factors contribute to the generation of a high-risk group. House holds contacts of subjects chronically infected with HBV are at very high risk due to repeated exposure and multiple modes of HBV transmission to them. Nearly 60% chronic liver disease in India is due to chronic HBV infection and 25% of the HBV related cancer in India are due to mutant forms of HBV. The transmission of HBV mutant infection is also known. Due to prolonged and repeated exposure of close family contact, the spread of the mutant forms are likely to be more common. This study has identified some important epidemiological, clinical, virological and molecular aspect of hepatitis B virus infection in close family contacts of chronic liver disease patients in India.

One hundred fifty four histologically proven chronic liver disease patients (Index subject) and their 749 family contacts, who gave consent for the participation were enrolled in the study. Care was taken to include index patients who had at east four live members in the family and were living together for at least 5 years. Fifty HBV negative healthy subjects and their 210 house hold contacts were enrolled as control. Contacts of chronic liver disease patients and controls were interviewed thoroughly to find out the exposure to the risk factors, specially the horizontal or vertical mode of transmission. Serum specimen of every subject was analyzed for the presence of HBsAg, HBeAg, HBeAb and IgG anti-HBc using enzyme immuno assay, and a molecular marker of HBV infection, HBV DNA by polymerase chain reaction. The HBV marker studied in the contacts were used to identify subjects with (a) HBsAg +ve (ongoing infection) and (b) only IgG anti-HBc +ve (evidence of past exposure). The index patients or contacts who were found to be HBsAg positive was further studied by HBeAg testing to know the high viral replicative status. A complete profile of test of liver function was also done in each specimen. Liver biopsy was performed in contacts with positive HBV DNA after obtaining an informed consent. The features in the liver biopsy were graded to define the histological activity index.
Patients and contacts were categorized on serological data as follows: (a) HBsAg/ HBeAg positive (b) HBsAg/ HBeAb positive (c) HBsAg negative/ IgG anti -Hbc positive. To identify the viral mutants, eleven index patients and their contacts were randomly selected. This included six HBsAg/ HBeAg/ HBV DNA positive and 5 HBsAg/ HBeAb/ HBV DNA positive index patients. Serum sample of every contact of the index patients was studied for the presence of HBV DNA and if found positive, was taken region were sequenced by, direct sequencing of the PCR product in an automated sequencer ABI Prism 377 (Perkin Elmer USA). The sequences thus obtained were compared and aligned with the consensus sequences of Gene Bank using the Mult Alin software. The important observations of the study are highlighted below:

7.1 The present point prevalence study revealed that chronic HBV related disease is prevalent all over India, with a male predominance. More than one third (44%) of the patients were living in the joint family set-up, where more than one generation and related families lived in the same premises. In 35% of the families, number of members living together was more than six.

7.2 Index Patients: One fourth of the liver disease index patients had infection with HBeAg -ve / HBeAb +ve / DNA +ve virus. This mutant virus could be a pre core mutant virus. One fourth (23%) of the index chronic liver disease patients also gave a history of liver disease in the family.

7.3 Contacts: The prevalence of HBV infection in family contacts of chronic liver disease patients was significantly higher than the contacts of the healthy control. Around seventy five percent of the families were found to have at least one affected member (“a positive family”) which was significantly higher than the control group (75% vs. 22% p<0.05). Clustering (with more than one affected member in the family) of HBV infection was observed in 42% of the families and more than 4 affected members were present in 10% families.

7.4 Household contacts of chronic liver disease patients were at very high risk of acquiring HBV infection. Around 47% of the contacts had at least one marker of HBV infection. Among 749 contacts studied, HBsAg positivity was found in 18.3% and evidence of past exposure to HBV
(IgG anti-HBc) was found in 28% contacts after excluding HBsAg positives contacts. The difference was significant compared to the contacts of the healthy control group for both HBsAg positivity and IgG anti Hbc positivity (18.3% vs. 2.4% and 28% vs 11%).

7.5 Prevalence of HBsAg in the first degree contacts was two fold higher than in the second degree contacts and sexual contacts (p<0.05).

7.6 The prevalence of HBsAg was nearly constant (~ 19%) in age groups till the age of 40 years, after which it increased to 25%. However a pattern of increase in the IgG anti-HBc positivity was observed with age.

7.7 HBeAg and HBeAb was found to be positive in 32% and 31% of the HBsAg positive contacts respectively.

7.8 Forty seven percent of the HBsAg positive subjects were HBV DNA positive. All the (100%) HBeAg positive contacts were HBV DNA positive, however HBV DNA was positive in around 55% of HBeAb positive contacts.

7.9 HBV DNA determination is a better marker for the detection of HBV Infection. The study showed that in patients with HBeAg negative status HBV DNA positivity is an important marker to identify presence of ongoing HBV infection. Of the HBsAg positive contacts 31% were HBeAg negative / HBeAb positive, out of which of these nearly 55% were HBV DNA positive. This indicated ongoing replication and possibility of pre core mutant virus in the patients DNA studies were also helpful in HBsAg negative/ IgG anti-HBc positive contacts, the HBV DNA positivity was 61%, compared to 8% in contacts of healthy control (p<0.05). None of the HBsAg negative / IgG anti-HBc negative subjects were HBV DNA positive. These data reinforce the utility of HBV DNA determination for the detection of HBV infection, more so in identifying subjects with past exposure having viremia and potential to transmit HBV infection.

7.10 The routes of transmission of HBV were carefully studied HBV infection within family was found to be transmitted through horizontal, vertical and sexual routes. The study revealed horizontal, vertical and sexual transmission of HBV in 71%, 20% and 9% respectively. Only 6% HBsAg positive contacts gave a history of prior exposure to HBV.
7.11 Horizontal mode of transmission was the primary mode of transmission within families irrespective of the endemicity of the area and prevalence in general population. The present study shows a significantly high prevalence of HBsAg positivity in the first degree relatives and siblings as compared to the second degree and sexual contacts. This observation supports the horizontal transmission of the HBV infection. Studies done in family contacts from low, intermediate and high endemic areas documented horizontal mode as major mode of transmission.

7.12 Liver disease was common in the contacts of chronic liver disease index patients. One half (49%) of the HBsAg positive contacts of chronic liver disease patients had histological features of chronic liver disease. Wild type infection was found in 32% and possible pre core mutant infection in 17% HBsAg positive contacts. Among the contacts infected with wild type HBV, chronic active hepatitis was present in 84%, cirrhosis in 11% and hepatocellular carcinoma was found in 5% contacts. Among the contacts infected with precore mutant HBV, chronic active hepatitis, cirrhosis and hepatocellular carcinoma was observed in 67%, 12% and 20% respectively. Hepatocellular carcinoma was more often seen with HBeAb positive HBV infection. Three fourths of the chronic liver disease contacts were asymptomatic and were detected to have liver disease only during family screening.

7.13 HBV DNA analysis in HBsAg positive contacts showed presence of possible precore mutation (HBsAg/HBeAb/HBV DNA positive) in 17% contacts. In the HBsAg negative group possible surface mutant (HBsAg negative/IgG anti-HBC/HBV DNA positive) was found in 21% contacts.

7.14 HBV DNA sequencing analysis was found to be very helpful in the study of viral mutations. In the present study sequencing of the “S” gene region was carried out in 25 HBsAg positive and 8 (6 contacts of the chronic liver disease patient and 2 contacts of healthy control) HBsAg negative/IgG anti-HBc positive subjects. In the HBsAg positive group mutations were found to be present in the 84% (21/25). In the HBsAg negative contacts of chronic liver disease patients of the six specimens sequenced, mutations were found in 16% (1/6) subjects. Sequencing of precore region was carried out in 15 HBeAg positive subjects, 10 HBeAg
negative / HBeAb positive and 8 IgG anti HBc positive subjects (6 contacts of the chronic liver disease patient and 2 contacts of healthy control). All 10 (100%) HBeAb positive specimens had mutation in the precore gene, while none of the HBeAg positive or IgG anti Hbc positive subjects were found to have mutation in the precore region.

7.15 The most common mutations in the "s" gene included, T118V (48%) and A128V (41%). Other mutations found in the "s" gene were at amino acid 114, 117, 120, 124, 126, 127, 129, 132, 135, 137, 138, 142, 143 and 145.

7.16 Novel mutation in the "s" gene were found at C137stop codon and C138stop codon. However these mutations were observed in only one contact who was HBsAg negative / IgG anti-HBc positive.

7.17 Mutation of amino acid Glycine to Arginine at position 145 does not necessarily results in HBsAg negativity. This mutation was present in three of the 25 HBsAg positive specimens and one of the 8 HBsAg negative /IgG anti HBc positive specimens.

7.18 Mutation in the pre core region could result in HBeAg negative phenotype of HBV. Molecular analysis showed presence of precore mutation in 30% of the specimen and were HBeAg negative. The most common mutation in this region with HBeAg negative phenotype was found at W28stop codon in 60% samples. Other mutations of the precore region were at codon 25 and 29 (nt 1898).

7.19 Molecular epidemiology can be used as a tool to study horizontal transmission of HBV infection. Molecular analysis study showed ~ 100% sequence homology between index patient and its contact, thus an intrafamilial horizontal transmission of the HBV infection was confirmed.

Special features of the study

- The present study is the largest series of family contacts involving 154 liver biopsy proven chronic liver disease patients and their 749 close family contacts.
- For the first time detection of HBV DNA, a molecular marker of HBV infection, was used in family contact study. A total of nine hundred and three specimen were analyzed for the presence of HBV DNA.
- Around 17% of HBeAb positive contacts were highly infectious.
These contacts were HBV DNA positive with deranged serum ALT. Therefore it is important to know that subjects with HBeAb positive status with raised ALT levels should not be ignored.

Method of studying vertical transmission should not be based upon the detection of HBsAg, it should rather involve determination of serum ALT levels and HbeAb.

In the IgG antiHBc positive contacts of healthy control 8% were HBV DNA positive. The sequence analysis showed wild type virus. Hence today ALT and HBV DNA is the useful marker to detect HBV infection.

The study highlights the family contacts as a high risk group and nearly 50% contacts have liver disease.

The study has provided a unique opportunity to study emergence of spontaneous natural mutations in a cohort of family contacts.

Limitations of the study

The study has highlighted many important features of HBV prevalence, transmission, mutants, spectrum of liver disease in the close family contacts of HBV related chronic liver disease patients, which would help in planning vaccination strategies in the high risk group. However the shortcoming of the study are:

- The study group was predefined to be only limited to family contacts.
- It was not possible to identify precisely that which contact had acquired the HBV infection first.
- It was also difficult to determine the duration of exposure.
- Since it was a serological and molecular study, the natural history of patients infected with different types of HBV mutant virus was not studied.
- Clinical significance of the mutations found were not assessed.
- The role of other viral factors in the transmission, specially the HBV viral load remains to be studied. Problem of viral quantitation of wild and mutant type of virus not solved.

Future plans

We would like to study the significance of different viral
mutation by cloning the viral strain with most common mutation T118V and A128V and novel mutation C137 stop and C138stop codon of the “S” gene found in the family contacts. The study of gene expression of such mutant type of viral strain will help in the development of new diagnostic assays for the detection of mutant type of virus which are usually missed with conventional ELISA.

Recommendations

Keeping in view the results of the study, it is recommended:

- Family contact should be screened for all marks of exposure (HBsAg, IgG anti-HBc, anti HBe). If found positive for HBsAg then HBeAg should be determined. If found negative for HBsAG then IgG anti-HBc / HBeAb should be routinely done. In all positive contacts HBV DNA testing and serum ALT should be mandatory.
- Vaccination in family contacts of CLD patients should be mandatory.
- Elevated ALT levels should be taken as a high index of suspicion for underline liver disease.
- Further studies on the frequency of transmission of Hepatitis B virus should be carried out using molecular marker HBV DNA.
- Significance of transfusion associated Hepatitis from IgG anti-HBc positive healthy donors should be studied.
- Non familial close contacts are equally prone to acquire HBV infection as evidenced in one family. This suggests HBV more of environmental horizontal spread than genetic.
- Genotype of Hepatitis B virus should be studied.