Discussion
6. DISCUSSION

6.1 PREVALENCE PATTERN OF HCV IN ASYMPTOMATIC PREGNANT WOMEN

The prevalence of HCV around the world ranges between 0.2 - 2% and the infectivity rates ranging between 0.3 - 24.5%. The highest prevalence has been reported in Ukraine and in the Central African countries of Gabon and Cameroon and in Egypt. In Northern African and Arabian countries such as Libya, Yemen, Saudi Arabia and Ethiopia, prevalence rates range between 1.4 and 2.1%. High prevalence rates have also been reported in South East Asian countries such as Thailand, Malaysia and India (Khaja et al., 2002). Thus with approximately 170 million people worldwide estimated to be infected with HCV, a figure that is 4 times higher than the HIV infection status (Lauer et al., 2001), HCV has the potential to be the next pandemic.

Numerous studies have examined the prevalence of hepatitis C among pregnant women. The studies vary considerably in terms of the size of the study. In general the prevalence of detectable antibody to HCV in pregnant women ranges from 0.1% to 2.4% (Boxall et al., 1994; Ohto et al., 1994; Moriya et al., 1995; Resti et al., 1998; Conte et al., 2000; Ward et al., 2000; Goldberg et al., 2001; Caudai et al., 2003).

While reviewing available literature dealing with epidemiology of HCV in India, it became evident that though analysis of certain high-risk groups
and certain patient categories has been carried out, community based studies are almost lacking.

HCV prevalence data has been mostly derived from blood donors in India, with under representation of women and lack of participation by children and senior citizens. The rate of mother to infant HCV transmission is critical to predicting the burden of HCV infection in future generations.

In the background of this global scenario and the paucity of information on HCV prevalence in Southern India, the present study was conducted to document the level of HCV burden in antenatal women and mother to child transmission. The serum samples of antenatal women (n=3115) and babies born to HCV positive pregnant women (n=12) were tested by two commercially available ELISA kits namely, anti-HCV ELISA by Murex Diagnostics and anti-HCV ELISA by Xcyton Diagnostics. There was no differences seen in the results of both the ELISA kits. A cross section analysis of 3115 antenatal women for the prevalence of anti-HCV antibodies has shown a positivity rate of 0.57%.

An earlier study from India has reported HCV positivity in pregnant women to be 1.33% (Irshad et al., 1998). The higher positivity in that study could be attributed to the smaller sample size of 75 cases in contrast to the 3115 cases in the present study.

In comparison, studies by Boxall et al., 1994; Ohto et al., 1994; Marronconi et al., 1994; Moriya et al., 1995; Zanetti et al., 1995; Manzini et al., 1995; Resti et al., 1998; Okamoto et al., 1999; Heillemanns et al., 2000; Conte et al., 2000; Ward et al., 2000 and Goldberg et al., 2001 have
reported prevalence of anti-HCV positivity ranging between 0.14-2.4%. All the studies included at least 3000 subjects. The mean prevalence of anti-HCV in the 6 studies consisting of greater than 10,000 subjects was 1.26%, whereas in studies consisting of 3,000-5,000 subjects the prevalence ranged between 1.4-0.7%. Thus the results of the present study are in coincidence with that of previous reports outside India.

6.2 THE HCV CARRIER RATE AMONG THE PREGNANT WOMEN

The HCV carrier state among the pregnant women varies considerably in terms of the size of the study, geographic variables and adequacy of laboratory testing. The mean prevalence of anti-HCV in studies consisting of more than 10,000 women was 1.26% with approximately 60% of anti-HCV women having detectable HCV-RNA.

Zanetti et al., 1995, screened 21,516 pregnant women, in which 1.2% of them were anti-HCV positive and the HCV-RNA was 55%.

Resti et al., 1998, reported 1.5% anti-HCV positivity and 68% HCV-RNA positivity in 25,654 pregnant women screened.

Okamoto et al., 1999, reported the prevalence of anti-HCV in pregnant women to be 0.58% and 66% of them were HCV-RNA positive.

Major studies conducted with about 5000 subjects, the HCV-RNA positivity in pregnant was observed to be 70% (Manzini et al., 1995), 57% (Hillemanns et al., 2000) and 75% (ward et al., 2000).
In our study, the HCV-RNA pattern in anti-HCV positive women was found to be 44% (8/18), which is comparable to that of previous studies mentioned above.

6.3 RATE OF HCV TRANSMISSION FROM MOTHER TO INFANTS

Maternal and child health issues relating to hepatitis C virus (HCV) infection have recently assumed greater importance than ever before. From the Paediatric perspective, the availability of effective screening methods for HCV has virtually eliminated new cases of transfusion-associated hepatitis in children. Consequently, childhood acquisition of HCV infection through mother-baby transmission has become the most important mode of spread. It has been difficult to determine the rate of mother to baby transmission, partly because of reports of mother to baby transmission of HCV has been based on small numbers of patients, with differing disease definitions, with different study designs and using different assay systems. These reports tended to be heterogeneous and conflicting. Moreover, factors that promote mother to child transmission and the outcome of chronic HCV infection acquired by this route still require clarification. The reported rate of mother to baby transmission ranges between 0% to 35.3% among children born to anti-HCV positive women (Yeung et al., 2001).

In infants of mothers who are HCV-RNA seropositive and HIV seronegative, studies have reported rates of transmission from 0% to 15% with a mean rate of 4.7% (Roudot-thoraval et al., 1993; Zanetti et al., 1995). The rate of vertical transmission is higher (5% to 36% with a mean of 16%) when the mother is both anti-HCV and anti-HIV seropositive.
A correlation exists between higher maternal titers of HCV-RNA and the probability of mother to baby transmission. Although mother to infant transmission occurred across a wide range of maternal viral titers, it corresponded to a greater tendency for mother to baby transmission (Moriya et al., 1995; Spencer et al., 1997; Okamoto et al., 2000; Ruiz-Extremera et al., 2000) and in some studies there was no difference (Garland et al., 1998; Granovsky et al., 1998; Mazza et al., 1998; Resti et al., 1998; Ketzinel-Gilad et al., 2000).

In our study, all the babies born to anti-HCV positive pregnant women screened were anti-HCV negative and the rate of HCV-RNA transmission from mother to baby was found to be 11% (2/18) i.e., 2 infants acquired the infection from 18 anti-HCV mothers studied, but the rate of HCV-RNA transmission from mother to infant was 25% if the denominator is restricted to HCV-RNA viraemic women.

No significant differences were detected among PCR-positive and PCR-negative pregnant women in terms of the presence of Jaundice, serum bilirubin serum ALT and AST levels. Spontaneous loss of serum HCV-RNA, interpreted as either clearance of mother to baby HCV infection or transient viraemia, has been suggested in many studies (Lam et al., 1993; Garland et al., 1998; Ruiz-extremera et al., 2000; Conte et al., 2000). In these babies serum HCV-RNA was detected on at least one occasion and then the babies was subsequently found to be HCV-RNA negative.

In the present study, the two babies had persistent viraemia from birth to 12 months, inspite of the absence of clinical symptoms. This tendency to chronicity has also been reported previously (Ercilla et al., 1994).
Seronegative HCV infection (absence of a serologic response to HCV polypeptides) is a well-known entity. Thaler et al., 1991 reported all eight children studied born to anti-HCV and HCV-RNA positive mother to have HCV-RNA on several occasions during their first year of life, but none of these children actively produced anti-HCV antibodies. Similar findings were also reported from Japan (Kuroki et al., 1991) and Europe (Novati et al., 1992).

Our study correlates well with the above studies because the two babies who were HCV-RNA positive were anti-HCV negative from birth till 9th month of follow-up. However one baby demonstrated anti-HCV seroconversion in the 12th month.

6.4 HCV GENOTYPE PATTERN OF HCV-RNA POSITIVE MOTHER AND CHILD PAIRS

Over the past few years with the development of commercial assays for the identification of HCV genotypes, an impressive number of studies have investigated the molecular epidemiology of HCV infection worldwide and possible effects of HCV genotypes on the pathogenesis and therapeutic outcome in hepatitis C infection.

Because of geographical clustering of distinct HCV genotypes, genotyping is a useful tool for tracing the source of an HCV outbreak in a given population. Suspected non-conventional routes of HCV transmission could also be investigated by molecular analysis of HCV strains from different individuals. These include the vertical and sexual route of transmission.
HCV genotypes seem to show distinct geographic distribution. HCV genotypes 1a and 1b are prevalent in the United States; genotypes 1b, 2a and 2b are common in Japan; genotypes 1b and 2a in China and genotypes 3, 1 and 4 in India. Genotypes 1, 2 and 3 are seen in Europe and Australia. Genotype 4 is seen in North Africa and Middle East. Genotype 5 is seen in South Africa; and type 6 in Hong Kong.

There have been few studies investigating the distribution of genotypes in Indian patients with chronic liver disease as well as in blood donors. A study conducted among southern Indian individuals with a diagnosis of non-A, non-B hepatitis revealed the predominance of genotype 1 over genotype 3 (Valliamai et al., 1995).

Genotype 2b was the most frequently encountered in Chandigarh whereas 2b and 3 were detected in almost equal proportion in Delhi (Kar et al., 2000).

Panigrahi et al., 1996, sequenced HCV strains from 11 patients with chronic liver disease from northern India of these, three were infected with genotype 1, one was found to have mixed infection with genotypes 1 and 3; three patients were infected with genotype 3 and the remaining four isolates were grouped into a new subtype 3g. In a study conducted by Sukanya et al., 2003, genotype 1 was found to be frequently encountered in patients from southern India, whereas 76% of all genotype 3 strains were documented from eastern India. The predominance of genotype 3 in India has been recently corroborated in another study on 153 HCV strains (Das et al., 2002). In India, the occurrence of genotype 4, previously believed to be restricted to Middle East and Africa, is being observed.
Although there are considerable reports on the distinct geographical patterns and prevalence of various hepatitis C genotypes worldwide, studies to evaluate the effect of hepatitis C genotypes on mother to baby transmission of virus have been limited and scanty particularly in India. To the best of the author’s knowledge the present study is the first of its kind to evaluate the effect of hepatitis C genotype in mother to baby transmission of HCV in India.

The earliest studies on transmission of HCV from mother to baby and its genotype was by Ohto et al., 1994 in Japan, who reported transmission of identical HCV genotypes 1b and 2a from 3 mother-baby pairs. Zuccotti et al., 1995 reported subtypes 1b and 3a to be the most commonest HCV genotypes transmitted from mother to babies in Italy. Studies on mother to baby transmission of HCV and its genotypes by Matsubara et al, 1995, revealed that type 1b was the most prevalent followed by 2a and 2b.

Kumar et al., 1998, reported the role of breast-feeding in transmission of HCV from mother to baby and the prevalence of genotype 3a in these infants, which was consistent with each mother.

In the present study, 3115 asymptomatic pregnant women were screened for anti-HCV, of which 18 were anti-HCV positive. RT-PCR analysis of serum samples of these subjects revealed 8 mothers to be positive for HCV-RNA and HCV-RNA transmission could be observed in only 2 mother-baby pairs after follow up studies for one year. Genotype studies of these pairs revealed 1a and 4 mixed genotypes, which was concordant with each mother / baby pair.
The report on prevalence of genotype 1a is consistent with the reports of previous studies on HCV genotypes in India. However, the prevalence of the genotype 4 is not well established in India albeit a few sporadic reports like Sukanya et al., 2003 in chronic hepatitis.

Although in our study we have reported the prevalence of mixed genotype 1a and 4 infection in mother and baby, there have been reports of mixed genotype infection in mother and single genotype infection in baby, like Aizaki et al., 1996. The presence of selection pressure due to 12-nucleotide insertion to a single genotype during transmission was suggested in this study. The biological significance in such kind of transmission needs to be studied in detail in future.

6.5 MOLECULAR SEQUENCING OF THE HCV GENOTYPES AND COMPARISON WITH THE RESULTS OF LINE PROBE ASSAY

The gold standard for genotypes assays is nucleotide sequencing of the viral genome. Since entire genomic sequence is difficult to perform and laborious, it is apt to sequence certain sub-genomic regions. The regions normally used for genotype sequencing are 5' UTR region, NS5B region, core region, NS3, E1 and E2 regions.

Regarding the nucleotide sequence comparison of the mother and baby pairs, various authors outside India, has sequenced various sub-genomic regions.

Weiner et al., 1993, sequenced the putative envelope glycoprotein 72 (gp72) hypervariable domain (E2 HV) from a mother-infant pair to assess the
distribution of HCV variants in three mothers and one mother-baby pairs. The data indicate that (i) quasi species distributions of ECV E2 HV variants were found in all four mothers. (ii) a single predominant HCV E2 HV variants was found in the baby of a mother shown to have nine predominant E2HV variants and (iii) the babies E2 HV variants was highly related to, but not identical, with the nine variants identified in the mother at the time of birth.

Lin et al., 1994, compared the nucleotide sequences (310 bp) from the NS3 region of the HCV genome between the mother-baby pair (n=1), which showed a homology of 99.4% of the mother - baby pairs. These data indicated that the baby was indeed infected by HCV perinatally by her mother.

Ohto et al., 1994, sequenced the core region of HCV from seven mothers - baby pairs. The genomic sequence of HCV of the babies was almost identical (97% homology) to that from the mother.

In order to prove the mother to child transmission in mother - baby pair, Aizaki et al., (1996) sequenced and compared the nucleotide and deduced amino acid sequence of the hypervariable region (HVR) of the envelope glycoprotein E2 from the HCV clones. The amplified cDNA fragments of the expected size were subcloned into the M13 sequencing vector plasmid. Nucleotide sequences of the 10 clones from the serum samples from the mother and the baby were determined by the di-deoxy nucleotide chain termination method. In the one-mother baby pair analyzed for sequencing, the mother was found to have mixed infection with type 1a and 1b. However, the Hepatitis C virus genome obtained from the baby was only from type 1b.
Genotyping studies by sequencing in India have so far targeted HCV infection in blood donors and in chronic liver disease subjects and no major study has so far addressed HCV genotypes involved in mother to child HCV transmission.

In the present study, the two mothers - baby pairs, which were analyzed with Innolipa Hybridization assay for genotypes have been subjected for sequencing. The individual nucleotide sequences of the cloned PCR products from mother baby pairs were subjected for direct sequencing and compared with EMBL data base using BLAST alignment search tools. The sequence analysis of our study revealed the existence of the same genotypes 1 a & 4 in both mother - baby pairs, which had 96% homology between the first mother / baby pair and 99% homology between the second mother / baby pair.