Review of Literature
2. REVIEW OF LITERATURE

Hepatitis C virus is a causative agent of what was formerly known as "Parenteral type non-A, non-B hepatitis". Once thought to be a disease of little significance, HCV is now recognized as the most common cause of post transfusion and chronic viral hepatitis in the western world and ranks second to chronic alcoholism as the cause of cirrhosis, end stage liver disease, and hepatocellular carcinoma in the United States. Infection by HCV is often silent, only discovered by serologic screening during the blood donation or routine health screening. The recent discovery of the HCV genome in 1989, has led to the development of diagnostic tests and a better understanding of the natural history of this disease (Choo et al., 1989).

Vertical transmission is the most frequent mode of HCV acquisition in pediatric setting and its epidemiological impact will increase in the future as a consequence of the decline of post transfusion HCV hepatitis.

The symptomatic disease leading to jaundice in pregnancy may be caused by any of the five hepatitis viruses, designated as Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Hepatitis D virus (HDV) and Hepatitis E virus (HEV). The clinical expression of jaundice in pregnancy includes (a) Acute viral hepatitis, (b) Sub-acute hepatic failure and (c) Fulminant hepatic failure. While hepatitis A and E viruses always bring about symptomatic liver disease in pregnancy, asymptomatic viral infection has been demonstrated significantly in hepatitis B virus infection and in hepatitis C virus infection.
2.1 HAV IN PREGNANCY

The incidence of acute hepatitis A (HAV) in pregnancy is < 1:1,000. HAV infection whether merely gastroenteritis or overt and obvious hepatitis, can cause dehydration and hypovolemia in the gravida, thus predisposing to decreased uterine blood flow and increased myometrial activity. There are no reports on intrauterine HAV infection or any teratogenic effects of the virus on the fetus. Therefore, a major life threatening aspect of the infection to the baby is preterm birth or decreased placental perfusion, depending on the degree of severity of the maternal illness. Acute hepatitis during pregnancy fails to demonstrate fetal transmission of HAV, although an increase in preterm deliveries may occur. Nevertheless, acute maternal HAV infection near the peripartum period poses a threat to the neonate. It is recommended that infants born to a mother who developed acute HAV infection within 2 weeks of delivery receive an intramuscular injection of 0.5ml of inactivated whole-virus HAV vaccine. Measures also should be taken to minimize faeco-oral spread of virus from the infected mother to her newborn and within the nursery. Antepartum and intrapartum transmission from an infected mother has not been so far reported and pregnant women with hepatitis A generally are not thought to transmit HAV to their offspring (John *et al.*, 1993).

2.2 HBV IN PREGNANCY

Hepatitis B virus (HBV) causes transient and chronic infection of the liver. The consequences of Hepatitis B are extremely variable. The incubation period may range between 6 weeks and 6 months. The infection may be characterized by, (1) Acute illness with jaundice followed by
recovery, (2) a sub-clinical infection followed by recovery, (3) an acute illness that progresses to chronic hepatitis, (4) a sub-clinical infection followed by chronic hepatitis or, (5) a fulminant fatal disease.

2.3 ACUTE HEPATITIS B IN PREGNANCY

Acute Hepatitis B is an unusual complication of pregnancy and often results in premature labour. If the acute disease occurs in the third trimester of pregnancy, there is 50-70% of chance that the infant might become HBsAg positive (Schmeitzer et al., 1973; Tong et al., 1981, 1985; Gupta et al., 1986). Acute hepatitis B resembles all other forms of acute viral hepatitis clinically and cannot be identified specifically be features of history, physical examination or by liver chemistry profile.

2.4 CHRONIC HEPATITIS B

Some forms of chronic hepatitis B infection follows an acute disease course in 5-10% of cases. Most of these patients are symptomatic carriers, but others have low-grade chronic persistent hepatitis or chronic active hepatitis with or without cirrhosis.

Chronic HBV infection is not a static disease but is dynamic, if followed-up over a long period of time. An individual can shift from one state to the other and back, more than once. A so called 'carrier' could change into active liver disease at any time without any symptoms or signs. Hence it is suggested that the term 'carrier' should be deleted from the terminology of hepatitis B and should be replaced by the term 'chronic hepatitis B virus infection' (INASL, 1999).
2.5 HBV TRANSMISSION IN INDIA

India is estimated to have 43 million carriers of hepatitis B, which works out to be about 10% of the world population. The estimated national average of HBV prevalence is 4%. The HBsAg prevalence in pregnant women in India is 2.2-5%. The HBeAg positivity rate in HBsAg positive pregnant women is 6-24%. Hepatitis B "e" antigen positive chronic carriers transmit hepatitis B to 80-100% of the infants. This frequency of transmission is much lower (0-40%) if the mother is not hepatitis B "e" antigen positive. When the mother has already developed antibody to this antigen (anti-HBe) the observed transmission rates have usually been less than 10%. While 40-50% of pregnant Asian hepatitis B carriers are positive for Hepatitis B "e antigen", this percentage is lower (0-10%) in Western populations (Chin et al., 1993).

2.6 HEPATITIS C VIRUS

Hepatitis caused by Hepatitis C virus (HCV) has become a major emerging infectious disease problem, with an estimated 170 million people infected worldwide and thus represents a viral pandemic, which is five times as widespread as infection with the human immunodeficiency virus type 1 (HIV-1) (Lauer et al., 2001).

2.7 HISTORY

Treatment of haemophiliacs with Factor VIII concentrates (F VIII) is known to be associated with a high risk of PT-NANB hepatitis, presumably due to the inclusion of one or more infected donor plasma units in the large
pools of plasma used by commercial firms to prepare F VIII concentrates (Craske et al., 1975). The early identification of three lots of F VIII concentrate implicated in the transmission of disease to two individuals in 1977 led to the recovery of proven-infectious materials, which was subsequently used for identification of virus-like particles by immune electron microscopy and possible transmission of disease to nonhuman primates. The cloning of HCV was initiated by ultracentrifugation of large volumes of chimpanzee plasma that had been shown to have an unusually high infectivity titre (10⁶ chimp infectious dose/ml). Nucleic acid was then extracted from the centrifuged pellet. A denaturation step was included before the synthesis of complementary DNA (cDNA), so that either DNA or RNA could serve as a template.

Conversion of RNA into cDNA involved reverse transcription using random primers. The resultant cDNA was inserted into a cloning vector λgt11, and expressed in E. coli (Choo et al., 1989). After lysis of the bacteria, expressed proteins that had adhered to an overlying filter were immunoscreened with serum from a patient who had NANB hepatitis and then with radiolabelled antoglobulin. A single clone (5-1-1) among millions tested was reactive by autoradiography. A larger clone, c-100-3, was assembled from several overlapping clones and expressed in yeast as a fusion protein using human superoxide dismutase (SOD) to facilitate expression. The c-100-3 antigen has become the basis of licensed solid-phase immunoassays that detect the complementary antibody in HCV carriers (Kuo et al., 1989; Bradley et al., 1991).
2.8 STRUCTURE AND GENOMIC ORGANIZATION OF HEPATITIS C VIRUS

HCV is a RNA virus with a positive sense, single-stranded genome of approximately 9,400 nucleotide encoding a single polyprotein of approximately 3,000 amino acids. The long open reading frame (ORF) is flanked at each end by a short untranslated region (UTR). The genome structure is most similar to viruses of the family Flaviviridae, which includes many of the arthropod-borne viruses. As in other flaviviruses, the three N-terminal proteins of HCV (core, envelope 1, and envelope 2) are probably structural, and the four C-terminal proteins (nonstructural 2,3,4, and 5) are thought to function in viral replication.

The 5' UTR is a highly conserved region of 341 nt and has a complex secondary structure. It contains an Internal Ribosome Entry Site (IRES) and presumably is important in the translation of the long ORF. The 3' UTR contains a short region that varies in sequence and length, followed by a polypurimidine stretch of variable length, and finally a highly conserved sequence of 98 nt, which constitutes the terminus of the genome. The function of the 3' UTR is not known, but is thought to be essential for viral replication.

The envelope 1 (E1) and 2 (E2) regions of HCV are the most variable regions within the genome at both the nt and amino acid levels. A 27 amino acid hypervariable region of the E2 protein (HVRI) has been described that may be the mechanism by which the virus evades the host's immune system.
The nonstructural regions 2 (NS2) and 3 (NS3) contain a Zn-

dependent protease that cleaves the polyprotein at the NS2-NS3 junction.
The aminoterminal portion of the NS3 protein also has proteinase activity
and cleaves the polyprotein at several sites. The carboxyterminal portion of
the NS3 protein has helicase activity, which is important for HCV replication.
The NS4A protein is a cofactor for NS3 proteinase activity. The NS5B region
encodes the RNA-dependent RNA polymerase, which replicates the viral
genome (Major et al., 1997) (Fig- 2.8).

Broadly, HCV transmission modes have been divided into two major
categories:

1. Parenteral Transmission: Transfusion recipients, plasma product
recipients, haemodialysis patients, organ transplant recipients,
intravenous drug users and health care personnel.

2. Non-Parenteral Transmission: Vertical, sexual, interfamilial and
nosocomical transmission.

2.9 ACUTE HEPATITIS C

Acute HCV infection, in general is relatively mild with only 20-30% of
infected persons developing symptoms or clinically evident acute hepatitis C.
However, 70-80% of acute HCV infections does not resolve and result in a
persistent viral infection. Symptoms and jaundice have been more frequently
reported in community-acquired disease. Jaundice is often preceded and
accompanied by fatigue, lethargy, myalgia, low-grade fever, nausea,
vomiting and right upper quadrant pain or discomfort. This clinical syndrome
occurs within 2-26 weeks of exposure and last for 2-12 weeks.
Hepatitis C virus (HCV): model structure and genome organisation

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Fig-2.8
Elevation of aminotransferases usually occurs after the 4th week, but may also occur as early as the second week following exposure. HCV-RNA becomes detectable by sensitive PCR in serum 7-21 days after exposure and then titres fluctuate. Broadly specific antibodies appear within 20-150 days with a mean of approximately 50 days.

2.10 CHRONIC HEPATITIS C

Patients with chronic HCV often have no symptoms but may complain of non-specific symptoms such as fatigue, muscle aches, anorexia, right upper quadrant pain and nausea. Cirrhosis occurs in less than 20% of the patients with hepatitis C, usually becoming detectable in the second or third decade after infection. An undermined proportion of chronic infections are asymptomatic with normal liver enzymes and relatively normal liver histology. Factors that influence the rate of progression of chronic hepatitis C to cirrhosis and hepatocellular carcinoma include alcohol abuse, age at time of infection, liver histology at initial biopsy and possibly viral titre. The role of other factors, such as viral genotype, co-infection with hepatitis B virus (HBV) or HIV, gender and immunogenetic factors, is less well understood.

2.11 DIAGNOSIS

The diagnosis of HCV infection is done by either the exclusion tests and surrogate markers testing or the much reliable HCV antibody and RNA testing.
2.11.1 Surrogate markers

Initial reports have stressed the importance of ALT testing and anti-HBc IgM testing for the diagnosis of HCV infection, as elevated ALT levels and anti-HBc IgM positivity correlated well with anti-HCV positivity (Alter et al., 1981). Later studies have shown the insignificance of these surrogate marker testing and have shown anti-HCV to be a reliable marker (Barrera et al., 1991).

2.11.2 Liver biopsy findings

Liver biopsy findings are useful in understanding the severity of liver damage. Various degrees of liver damage ranging from persistent or active chronic hepatitis to cirrhosis can be evidenced. The severity of the liver damage may be graded with "Knodell's histologic activity index". Four types of liver lesions can be observed; periportal necrosis (score from 1-10), intralobular degeneration and focal necrosis, portal inflammation and fibrosis (each one with scoring 1 - 4). A single biopsy may not be representative of the overall activity of infection and liver damage. However, ALT levels and liver biopsy findings may not pinpoint the specific viral etiology as due to HCV.

2.11.3 Hepatitis C antigen detection

Visualisation of complete HCV virions has been extremely difficult primarily because of the low titre of the virus in clinical samples and this has a direct effect on antigen detection as even the antigenaemia is equally low. Currently, HCV antigens are detected only in the liver biopsies by using
immunohistochemical techniques (Infantolino et al., 1990) or by immunoperoxidase electron microscopy (Shimizu et al., 1995). These tests are done at a research level and there are no commercial antigen-detection systems yet available for HCV.

2.11.4 Hepatitis C antibody detection

Two testing patterns are available for the detection of anti-HCV antibodies. They are

(a) Screening tests for anti-HCV (Enzyme immunoassay-EIA) and
(b) Supplemental antibody tests (Recombinant immunoblot assays-RIBA)

2.11.5 HCV-RNA detection

Direct tests for HCV antigens in serum are not available. So currently the detection of HCV genome (HCV-RNA) is considered to be the most reliable and direct marker for viral replication and infectivity of the virus.

Assays based on molecular detection of HCV-RNA tests are based on the PCR technique and have a lower limit of detection of fewer than 100 copies of HCV-RNA per mL of serum (50 IU/mL). Testing for HCV-RNA is a reliable way of showing HCV infection and is the most specific test of infection. A qualitative PCR assay is especially useful when transaminase concentrations are normal, when other causes of liver disease are present (ie, alcohol consumption), in immuno-suppressed patients (ie, graft recipients, HIV co-infected patients), and in acute hepatitis C before
antibodies have developed. Quantitative determination of viral RNA levels are necessary to assess the progression of the acute and chronic hepatitis due to HCV and to identify and monitor patients who are put on interferon therapy. Through quantitative testing, HCV-RNA levels can be monitored periodically in response to therapy. The baseline levels of HCV-RNA are to be determined before starting the treatment. Quantitative testing is also useful to recognize nonresponders and HCV relapse, and facilitate understanding of natural history and pathogenesis of HCV. The different types of quantitative HCV-RNA detection assays are Roche Amplitcor HCV Monitor assay, Branched-DNA signal amplification assay, Nucleic acid sequence based amplification (NASBA), Real-time detection system, Competitive RT-PCR assay.

2.12 TREATMENT

A number of trials suggest that early antiviral therapy with interferon can reduce the risk of chronicity (Omata et al., 1994). Pilot studies suggested that interferon alpha (α-IFN) was of potential benefit for patients with chronic hepatitis C infection (Davis et al., 1989). Within a few days of starting therapy a decrease was noted in the transaminase values. It also appears that levels of viraemia and genotype may be important in determining the response to treatment. Genotype 1b is particularly associated with a poor chance of recovery (Hopf et al., 1996). Several treatment regimens have been assessed in large trials, the first of which (standard interferon regimen monotherapy with three injections three times a week) was approved in 1990 and the last (combination of ribavirin and pegylated interferon) in 2002. The specifics of these treatments are: standard interferon α (α-2a or 2b) 3 MU three times a week for 24 weeks
and then 48 weeks; (Myers et al., 2002) a combination of standard interferon (3 MU three times a week) and ribavirin (1000-1200 mg per day) for 24 weeks or 48 weeks; (Poynard et al., 1998; McHutchison et al., 1998; Poynard et al., 2000) pegylated interferon for 48 weeks (α-2a 180μg, or α-2b at three doses: 0.5μg, 1.0μg, or 1.5μg per kg); (Zeuzem et al., 2000; Heathcote et al., 2000; Lindsay et al., 2001) and 48 weeks combination pegylated interferon and ribavirin (different doses of pegylated interferon and ribavirin) (Manns et al., 2001; Fried et al., 2002; Hadziyannis et al., 2002). Combination therapies have always been more effective than interferon monotherapy and pegylated interferon monotherapy. Antiviral therapy is monitored by quantifying the serum levels of HCV-RNA before and after the treatment.

2.13 PROPHYLAXIS

It is currently believed that HCV elicits a neutralizing antibody presumably directed against epitopes in the envelope proteins. This humoral immune response appears to be restricted and isolate-specific (Farci et al., 1994). The genetic heterogeneity of HCV with six clades, 11 genotypes and at least 100 subtypes recognized appears important. The immune response to infection by one genotype may be insufficient to overcome the risk of superinfection by other HCV genotypes (Farci et al., 1992; Kao et al., 1993). HCV vaccine development began with the use of recombinant HCV proteins as the immunogenic material (Choo et al., 1994). The initial candidate HCV vaccine, derived from the envelope glycoproteins (gpE1/E2) of HCV, with MF59 and muramyl dipeptide adjuvants, induced high levels of neutralizing antibodies in chimpanzees and provided protection in a proportion of animals challenged with low doses of the homologous strain (Choo et al.,
1994; Houghton et al., 1997). A recently suggested novel approach calls for the use of immune stimulating complexes containing the HCV core protein, which is well conserved among the various HCV genotypes and subtypes, as an adjuvant for the envelope glycoprotein vaccine (Polakos et al., 2001). Other approaches to vaccine development have included the incorporation of HCV proteins into recombinant viruses (Siler et al., 2002; Brinster et al., 2002). In one DNA vaccine study - utilizing chimpanzees, a plasmid DNA encoding the cell-surface E2 HCV protein was used as the immunogen. Antibodies and cell-mediated responses to E2 were elicited but on challenge with homologous HCV, sterilising immunity could not be achieved. Nonetheless, liver injury and viraemia resolved in the vaccinated animals, whereas the infection became persistent in the single non-immunised chimpanzee (Forns et al., 2000). Once an effective, safe vaccine is available, it seems likely that it initially would target groups at high-risk for HCV infection: namely users of illicit injection drugs, renal failure patients who are candidates for long-term haemodialysis, patients with blood-clotting disorders, sexual partners of patients with HCV infection, healthcare workers regularly exposed to blood and newborn of HCV-infected mothers.

2.14 HCV GENOTYPES

The first full-length HCV sequence was derived from a chimpanzee infected with a contaminated American F VIII concentrate. This sequence became known as the HCV prototype, HCV-US or HCV-1, to which each new sequence is compared (Houghton et al., 1990; Choo et al., 1991). As additional genome sequences from isolates from different parts of the world were determined and compared, it was evident that HCV exists as distinct genotypes with as much as 35% sequence diversity over the whole viral
genome (Okamoto et al., 1992). Sequence analysis of variable regions of the HCV genome has been used to investigate outbreaks of infection and to study modes of transmission. Two large outbreaks of infection associated with contaminated lots of anti-rhesus D immunoglobulin in Ireland and Germany were investigated using molecular typing (Hohne et al., 1994; Power et al., 1995). In both studies, sequence analysis showed that the virus infecting women was the same as that found in the implicated batches of anti-D. In another report, sequencing of a region of NS3 provided evidence of patient-to-patient transmission during colonoscopy (Bronowicki et al., 1997). Sequence analysis is also becoming a routine part of investigations of HCV infections associated with blood transfusions (Cantaloube et al., 2001).

In addition, molecular analysis has been used to study vertical and sexual transmission of HCV (Weiner et al., 1993; Chayama et al., 1995; Healey et al., 1995; Aizaki et al., 1996). Much of the early literature on genotyping is confusing because investigators developed and used their own classification schemes. However, a consensus nomenclature system was developed in 1994. In this system, the genotypes are numbered using Arabic numerals in order of their discovery, and the more closely related strains within some types are designated as subtypes with lowercase letters. The complex of genetic variants found within an individual isolate is termed the quasispecies. The quasispecies results from the accumulation of mutations that occur during viral replication in the host.

Six major genotypes were originally identified. Sequence analysis of the E1 region suggested that HCV could be grouped into 6 major genotypes and 12 subtypes (Bukh et al., 1993). The same investigators sequenced 573 nt of the core region of the same isolates to confirm this classification scheme (Bukh et al., 1994). Simmonds et al., (1993) were also able to
classify HCV isolates into the same six major genotypes and numerous subtypes using sequence analysis of the NS5B region. Analyses of full-length ORF sequences have confirmed the original classification scheme based on analyses of individual gene regions (Bukh et al., 1995).

Genome sequence analysis of HCV isolates from Southeast Asia have led some authors to propose new major genotypes 7, 8, 9, 10, and 11 (Tokita et al., 1994, 1996). However, other investigators suggested that these variants could be classified within the six major genotypes originally described (Mizokami et al., 1996; Simmonds et al., 1996). Under this scheme, genotype 10 is a divergent subtype of genotype 3, and genotypes 7, 8, 9, and 11 are divergent subtypes of genotype 6. Over the past few years, with the development of commercial assays for the identification of HCV genotypes, a number of studies have investigated the molecular epidemiology of HCV infection worldwide and possible effects of HCV genotypes on the pathogenesis and the therapeutic outcome in Hepatitis C infection.

2.15 GENOTYPIC PREVALENCE

The distribution of different genotypes of HCV in the different regions of the world has been used to understand the evolution and epidemiology of this virus. In Europe, the relative prevalences of genotypes 1a and 3a in the population have increased over the last 40 years (Brechot et al., 1994; Pol et al., 1995). Genotype 1a the most prevalent type in North America, may have been imported to Europe from North America with blood products. Intravenous drug users are predominantly infected with type 3a, and its rise in prevalence may be tied to increased intravenous drug abuse (Fig- 2.15)
<table>
<thead>
<tr>
<th>Geographical Area</th>
<th>Main Genotypes</th>
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<tbody>
<tr>
<td><strong>America</strong></td>
<td></td>
</tr>
<tr>
<td>- USA and Canada</td>
<td>1a,1b,2a,2b,3a</td>
</tr>
<tr>
<td>- South America</td>
<td>1a, 1b, 2, 3a</td>
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<tr>
<td><strong>Europe</strong></td>
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</tr>
<tr>
<td>- Northern Europe</td>
<td>1a,1b, 2b,3a</td>
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<tr>
<td>- Western Europe</td>
<td>1a,1b,2a,2b,3a</td>
</tr>
<tr>
<td>- Southern Europe</td>
<td>1b,2c, (Italy, Spain)</td>
</tr>
<tr>
<td>- Eastern Europe</td>
<td>1b</td>
</tr>
<tr>
<td><strong>Asia</strong></td>
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<tr>
<td>- Turkey</td>
<td>1b</td>
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<tr>
<td>- Middle East</td>
<td>4</td>
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<tr>
<td>- China</td>
<td>1b,2a,2b</td>
</tr>
<tr>
<td><strong>Africa</strong></td>
<td></td>
</tr>
<tr>
<td>- Parts of northern Central Africa</td>
<td>4</td>
</tr>
<tr>
<td>- Egypt</td>
<td>4a</td>
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<tr>
<td>- South Africa</td>
<td>1,2,3,5a</td>
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<tr>
<td><strong>Pacific</strong></td>
<td></td>
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<tr>
<td>- Australia</td>
<td>1a,1b,2a,2b,3a</td>
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<td>- Taiwan</td>
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<tr>
<td>- Japan</td>
<td>1a,2a,2b</td>
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<tr>
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<td>1b, 2,3</td>
</tr>
<tr>
<td>- Vietnam</td>
<td>1b,2,6</td>
</tr>
</tbody>
</table>
2.16 HCV GENOTYPES IN INDIA

In India, mostly genotype 1b and 3 are predominantly found. In south India Valliammai et al., (1995) sequenced 24 HCV infected patients of which 21 showed HCV genotype 1 and other 3 were of HCV genotype 3. One isolate of genotype 3 (Isolate MN5) was found to be a new subtype of HCV that showed the sequence homology to an Indonesian isolate TD-3. Both these isolates were described to be a new subtype of HCV type 3 (Valliammai et al., 1995). Recently, a study in South India by Sukanya et al., (2003) sequenced 90 patients with chronic HCV infection for genotype determination. The genotype profile most frequently detected was genotype 3 followed by infection with genotype 1. Genotype 4 was seen in 5 patients and one patient had infection with HCV genotype 2.

In north India, Panigrahi et al., (1996) sequenced 11 HCV infected patients. Among the 11 sequenced samples, 7 samples were found to be genotype 3 and 3 samples were found to be of genotype 1; one sample was co-infected with 1 and 3 genotype.

2.17 METHODS OF HCV GENOTYPING

2.17.1 Nucleic Acid Sequencing

Analysis of amplified genomic sequences is the most definitive way to genotype HCV strains. The genotype is determined by sequence analysis of individual coding regions for which sufficient reference data are available such as 5’ UTR, E1, C, and NS5B (Bukh et al., 1992; Simmonds et al., 1993). Comparison of the unknown sequence with those of recognized HCV genotypes will identify the genotype of the isolate in question. Genotyping
schemes based on sequencing of variable genes such as E1, C, and NS5B provide enough resolution to determine types and subtypes. However, the 5' UTR is too highly conserved to discriminate all subtypes reliably (Smith et al., 1995). Genotyping methods targeting highly variable regions have higher failure rates because of primer mismatches and failed amplification reactions.

Sequencing reactions can be performed directly on PCR products or on cloned amplicons. Mixed infections with multiple genotypes may be missed with direct sequence analysis. Definitive detection of mixed infections requires analysis of a large number of clones. However, cloning may emphasize artifactual nucleotide substitutions or by selection during the cloning procedure (Foms et al., 1997; Smith et al., 1997).

The most common DNA sequencing method used is dideoxynucleotide-mediated chain termination. Recent developments in automation, throughput, and software have made nucleic acid sequencing increasingly accessible for clinical laboratories.

A standardised direct sequencing system was recently developed for routine clinical applications by Visible Genetics. The Trugene HCV 5'NC genotyping kit targets the 5'UTR (nt 96 to 282) and uses a proprietary single-tube chemistry that is robust and highly sensitive. This method can be used with the 244-bp amplicon generated by either the Roche Amplicor HCV or Amplicor HCV Monitor test as the sequencing template after a column-purification step (Ross et al., 2000). The sequencing chemistry produces bidirectional sequences using two different fluorophores to label the PCR primers. The software acquires the sequence data in real time, and each pair
of forward and reverse sequences is combined and automatically aligned with reference sequences stored in the library for type, subtype, and closet isolate determination. The library module contains approximately 200 sequences from the six major genotypes and 24 subtypes of HCV. The Trugene HCV 5'NC genotyping system appears to be a rapid and reliable means of determining HCV genotypes but, like all approaches targeting the conserved 5'UTR, cannot reliably distinguish all HCV subtypes (Ross et al., 2000; Halton et al., 2001; Ansaldi et al., 2001). Visible Genetics also has developed a kit for sequencing the NS5B region, but independent verification of its performance has not yet been published.

2.17.2 Hybridization

A reverse-hybridization assay was developed by Innogenetics (Ghent, Belgium) to genotype HCV. In this line probe assay (LiPA), biotinylated PCR product from the 5' UTR is hybridized under stringent conditions with 19 type- and subtype specific oligonucleotide probes attached to a nitrocellulose strip (Stuyver et al., 1993). Hybridizes PCR products are detected with a streptavidin-alkaline phosphatase conjugate. The second-generation assay discriminates among genotypes 1a, 1b, 2a/c, 2b, 3a, 3c, 4a-h, 5a, and 6a (Stuyver et al., 1996).

The results from the LiPA correlated well with results obtained by direct sequencing assays of 5' UTR and other genes in published evaluations but may not distinguish between genotypes 1a and 1b in 5% to 10% of cases and do not distinguish between genotypes 2a and 2c (Smith et al., 1995; Andonov et al., 1995; Zeuzem et al., 1995; Lau et al., 1996). The LiPA is the most common method used in clinical laboratories for HCV
genotyping because it can be used with amplicons from both qualitative of quantitative Amplicor HCV tests and is easy to perform and interpret. Mixed genotype infections are easily recognized by unusual patterns of hybridization with the typing probes. However, the LiPA requires a considerable amount of amplicon for typing, and the assay may fail regularly when the viral load is $<10^4$ copies/mL.

A DNA enzyme immunoassay (Sorin Biomedica, Saluggia, Italy) for HCV genotyping is based on hybridization of denatured amplicon for the core region to genotype-specific probes that are bound to the wells of a microtiter plate. Mouse monoclonal antibodies to double-stranded DNA are used to detect the hybrids. The results of the DNA enzyme immunoassay were highly concordant with the results of other genotyping methods in two evaluations (Viazov et al., 1994; Ross et al., 2000).

2.17.3 Subtype-specific PCR

In 1992, Okamoto et al., were the first to develop subtype-specific PCRs for determining HCV genotypes. First, the core region was amplified in an RT-PCR with a universal primer pair, and then a second round of amplification with a mixture of nested subtype-specific primers was performed. Each subtype-specific primer generated an amplicon of unique length. The amplicons were electrophoresed and the genotype was inferred from the size of the amplicon. Subtype-specific PCR overestimates the number of mixed infections because the primer designed for type 1b cross-reacts with some type 1a sequences, erroneously identifying mixed infections with 1a and 1b (Widell et al., 1994; Kleter et al., 1995; Foms et al., 1996; Smith et al., 1997). This method also has problems in determining the
genotype of isolates from Europe. The primer originally designed to amplify Japanese isolates of genotype 2a was unable to amplify sequences from a number of European isolates of genotype 2 (Giannini et al., 1995).

Other subtype-specific PCRs have been developed that target the NS5B (Chayama et al., 1993) and the core (Okamoto et al., 1996; Ohno et al., 1997) regions. The extent to which these assays overcome the limitations associated with the original subtype-specific PCR has not been determined.

Hu et al., (1998) described a novel genotyping method based on primer-specific and mispair extension analysis (PSMEA). This method depends on the unique properties of the 3' to 5' exonuclease proofreading activity of Pfu DNA polymerase. In the presence of an incomplete set of dNTPs, Pfu DNA polymerase is extremely discriminative in nucleotide incorporation and proofreading at the initiation step of DNA synthesis, completely preventing primer extension when mispairs are found adjacent to the 3' end of the primer. Using PSMEA and five type- and subtype-specific primers, it was possible to discriminate genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. This method was recently compared with four other genotyping methods for the detection of mixed genotype infections (Hu et al., 2000). PSMEA was the most sensitive method, detecting mixed infections in 7.9% of blood donors, 14.3% of patients with chronic HCV infections, and 17.1% of multiply transfused thalassemia patients with HCV infection.

Kreukulova et al., (2001) recently described a nested restriction site-specific PCR to detect and type HCV. This strain-typing assay takes advantage of the nucleotide sequence polymorphisms that occur at
restriction endonuclease recognition sites in the 5' UTR. It uses nested PCR, first to amplify a 661-bp segment spanning the 5' UTR and core regions and then five additional primers to generate multiple band patterns with the 661-bp segment as a template. The method generates a fingerprint pattern for the different genotypes without the use of any restriction endonuclease or other expensive reagents or equipment. The assay described in this report could reliably distinguish subtype 1b from the others but could be modified to differentiate any HCV genotype or subtype of interest.

2.17.4 DNA Fragment Length Polymorphism

Restriction fragment length polymorphism (RFLP) uses universal primers and nested PCR to amplify specific genomic segments and one or more restriction endonucleases to digest the amplicon at genotype-specific cleavage sites. The resulting DNA fragments are separated by gel electrophoresis, and each genotype is recognized by its specific digestion pattern. The NS5B and 5' UTR regions have been used for RFLP analysis to ensure complete digestion of the amplicons to avoid extra DNA bands that can lead to misinterpretation. Some HCV subtypes also cannot be identified accurately by RFLP analysis of the 5' UTR because of its high degree of conservation (Bukh et al., 1992; Smith et al., 1995).

A variation of RFLP uses another enzyme, Cleavase I (Third Wave Technologies, Madison, WI) to generate structure-specific cleavage products from a segment of the 5' UTR (Marshall et al., 1997). This method is called cleavase fragment length polymorphism (CFLP). It relies on the formation of unique secondary structures that result when DNA is allowed to cool after brief heat denaturation. These structures are the substrates for Cleavase I.
Because the formation of these secondary structures is determined by the nucleotide sequence, polymorphisms result in the generation of unique sets of cleavage products, or structural fingerprints, for each sequence analyzed. Genotype results determined with CFLP are reproducible and are highly concordant with results determined by DNA sequencing (Marshall et al., 1997; Sreevatsan et al., 1998).

2.17.5 Heteroduplex Mobility Analysis

Heteroduplex mobility analysis (HMA) depends on the formation of mismatches when two divergent DNA sequences are mixed, denatured, and allowed to reanneal. This process results in the formation of homoduplexes and heteroduplexes that migrate at different speeds during polyacrylamide gel electrophoresis. The mismatches reduce the mobility of the heteroduplexes through the gel, which are slowed in proportion to the sequence divergence. HMA has been used for both assessment of quasispecies (Wilson et al., 1995) and genotyping of isolates of HCV (White et al., 2000).

Genotyping by HMA involves mixing PCR products from viruses of unknown genotype separately with a panel of reference products of each genotype, then separating the heteroduplexes by electrophoresis. White et al., (2000) found that the pattern of heteroduplexes formed when 5' UTR amplicons from test isolates were mixed with the panel of reference isolates correlated with genotype as determined by sequencing and that HMA could identify mixed infections. HMA provides a rapid and inexpensive method for genotyping HCV that requires fewer resources than DNA sequencing but
requires access to a reference genotype panel and is not amenable to testing of large number of samples.

2.17.6 Serologic Genotyping

Genotype-specific antibodies directed against the immunodominant epitopes in NS4 have been used to develop serotyping or serologic genotyping tests. Two serologic genotyping tests are commercially available. An NS4 recombinant immunoblot assay was developed by Chiron (Emeryville, CA) that uses synthetic proteins from the NS4 and core regions to discriminate among HCV genotypes 1, 2, and 3 (Dixit et al., 1995). A second test was developed as a competition enzyme-linked immunoassay (Murex Diagnostics Ltd, Dartford, England). This assay uses eight branched synthetic peptides to detect genotype-specific anti-NS4 antibodies to discriminate among HCV genotypes 1 to 6 (Pawlotsky et al., 1997). The high degree of cross-reactivity among genotypes to these synthetic peptides necessitates absorption of the cross-reacting antibodies with an excess of heterologous peptides in solution before use.

Both of the assays described above lack sensitivity and specificity compared with direct sequencing or the LiPA for HCV genotyping. However, serologic genotyping is inexpensive and easy to perform and lends itself well to large epidemiologic studies. It is the only way to determine the genotype of a virus in patients with low-level viremia or those in whom the infection has been cleared and in specimens in which the RNA has been destroyed by improper handling.
Hepatitis C is a global health problem caused by infection with the HCV. Although representative prevalence data are not available from many countries. The estimated number of people with positive anti-HCV around the world is 170 million and approximately 75% of people with anti-HCV are chronically infected with the virus, the number of people with the infection may be approximately 127 million around the world. Since no data were available from 57 countries, the true number of people with HCV infection may be substantially higher. Although HCV is globally present, the prevalence pattern roughly parallels that of socioeconomic development. Lower prevalence estimates are reported from Western Europe, North America, and Australia. Countries at the higher end of the spectrum are found in Africa, Asia, and South America. The highest prevalence (17-26%) has been reported from Egypt (Kim et al., 2002) (Fig-2.18).

2.19 PREVALENCE OF HEPATITIS C AMONG PREGNANT WOMEN

Numerous studies have examined the prevalence of hepatitis C among pregnant women. The studies vary considerably in terms of the size of the study, geographic variables and adequacy of laboratory testing. In general, the prevalence of detectable antibody to HCV (anti-HCV) in pregnant women ranges from 0.1-2.4% (Resti et al., 2003). Early smaller studies from the United States found prevalence of anti-HCV between 4-5% (Reinus et al., 1992; Silverman et al., 1993). Small studies from Egypt reported a very high prevalence, between 10-20% (Agha et al., 1998; Kassem et al., 2000). A single study from Kinshasa (Democratic Republic of
Fig-2.18

Congo) contrasted the prevalence of anti-HCV in commercial sex workers with other pregnant women and found 6.6% and 4.3%, respectively. (Laurent et al., 2001)

Ohto et al., (1994), screened 7,698 pregnant women in which 53 (0.14%) of the women were positive for anti-Human Immunodeficiency Virus antibodies and 31 (58%) of them were HCV RNA positive. Three of the 54 babies born to these mothers (5.6%) became positive for HCV-RNA during the follow-up period.

Zannetti et al., (1995), screened 21,516 pregnant women of which 250 (1.2%) of the women were anti-HCV positive and HCV-RNA positivity was 55%. None of the babies born to HCV-RNA positive women were infected.

Resti et al., (1998), screened 25,654 pregnant women, in which 442 women (1.7%) were found to be positive for anti-HCV. 39 mother-child pairs dropped out and 403 completed the study. 68% (275/403) were found to be HCV-RNA positive. 13 children had acquired the infection and became HCV-RNA positive in the 275 HCV RNA positive women, showing the transmission rate of 5%. 6 children had HCV RNA immediately after birth.

Conte et al., (2000), evaluated 15,250 pregnant women among which 370 (2.4%) women were anti-HCV positive with 72% being HCV-RNA positive. A study conducted at St. Mary's Hospital, London by Ward et al., (2000), revealed 0.8% anti-HCV prevalence in 4825 pregnant women screened, with HCV-RNA positivity being 0.6%.
Similar study by Goldberg et al., (2001) in Ninewells Hospital, Dundee, observed 0.6% (23/3548) anti-HCV prevalence in pregnant women. Hepatitis C virus genotypes were analysed in all the above studies for RNA positive babies and their mothers. The mother and the baby pair had identical genotypes.

From these data it appears that the prevalence of anti-HCV antibodies among pregnant women is no different from that in the general age-matched population. Of the women with anti-HCV approximately two thirds may be expected to have active infection with detectable serum HCV women of child-bearing age, i.e., all women in the 15 - 45 year age bracket. Whether the fertility of women with chronic hepatitis C is identical to that of women without this chronic infection is also not established.

2.20 DEFINITION OF MOTHER TO INFANT TRANSMISSION

Many infants of mothers chronically infected with HCV are found to have detectable anti-HCV in their blood, which they have acquired through passive transplacental transfer of the IgG-antibody. Possible criteria for definition of mother to infant transmission of HCV infection include. (i) Detection of HCV-RNA in the infant by PCR in at least two serum samples during the first year of life (ii) detectable anti-HCV in an infant who is more than 18 months old. (iii) Confirming identical genotype between mother and the infant (Roberts et al., 2002).
2.21 ISSUES RELATING TO PREGNANCY AND CHRONIC HEPATITIS C

There are few reports regarding the effect of pregnancy on the clinical course of chronic hepatitis C, and the numbers of patients observed are generally small. In several series, a trend toward markedly lower or normal serum alanine aminotransferase (ALT) levels in the third trimester has been noted, with a return to pre-pregnancy levels in the first 3 to 6 months postpartum (Romero-Gomex et al., 1998; Conte et al., 2001). At the same time, a rise in serum HCV RNA levels has been detected (Gervais et al., 2000; Patemoster et al., 2001). The improvement in serum ALT levels may be caused by physiologic changes associated with pregnancy including expansion in plasma volume, high plasma concentrations of estrogen, and changes in immune reactivity. The extent of the reduction in serum ALT levels makes simple hemodilution an unlikely explanation (and a concomitant rise in HCV RNA levels is inconsistent). In a single case report, ALT levels normalized during pregnancy and serum HCV RNA concentration also dropped such that postpartum HCV RNA was undetectable is serum on 2 separate occasions 2 weeks apart: this was interpreted as spontaneous clearance of HCV infection (Zein et al., 2001). In some women with chronic hepatitis C, the liver disease appears to worsen with pregnancy. In one case, serum ALT levels normalized during pregnancy and HCV RNA levels dropped significantly in the third trimester; however, the patient experienced an acute flare of hepatitis with abrupt elevation of HCV RNA levels 1 month postpartum. Similar exacerbation of chronic hepatitis C has been reported among women from the United Arab Emirates (Kumar et al., 1998). In a small case-control study, liver histology was compared from before and after pregnancy in HCV-positive / Human Immunodeficiency virus (HIV) - negative women, and both increased inflammation and fibrosis were found (Fontaine
et al., 2000). Clearly, currently available data are inadequate to formulate general patterns of chronic hepatitis C disease course with pregnancy, but it is likely that individual variations in immune reactivity before and during pregnancy have an important role in determining the overall clinical course in the individual patient.

Observations regarding serum HCV RNA concentrations have also been highly variable. In some women, HCV RNA levels rise towards the end of pregnancy (Romero-Gomex et al., 1998; Gervais et al., 2000; Patemoster et al., 2001). The rise appears to be on the order of 50% above baseline. However, a few patients in one study were found to have undetectable serum HCV-RNA during pregnancy, becoming positive for HCV-RNA again in the postpartum period, (Romero-Gomex et al., 1998) and in another small study HCV-RNA levels fluctuated during pregnancy and became lower or undetectable in the postpartum period (Lin et al., 2000). If elevated viral levels are a risk factor for mother-to-infant transmission, then levels should be measured in the third trimester, because the measured concentrations of HCV-RNA at the onset of pregnancy may be neither representative nor predictive of the HCV-RNA concentration at the time of delivery.

Conversely, chronic hepatitis does not appear to have an adverse effect on the course of pregnancy or the birth weight of the newborn infant (Floreani et al., 1996; Hillemanns et al., 2000; Jabeen et al., 2000). The rate of spontaneous abortion was approximately the same as in the normal population. There was no increase in typical obstetric complications such as gestational diabetes and hypertension. In one study, pre-term delivery was quantitatively higher among anti-HCV positive women, but the difference has not statistically significant (Hillemanns et al., 2000). In the same series,
the rate of cesarean section was twice as high among anti-HCV positive women compared with the anti-HCV negative control group (statistically significant difference, p=.004), and this higher rate was attributed in part to the policy of the investigators not to use fetal scalp blood sampling for fetal surveillance when fetal heart rate is abnormal in anti-HCV positive women.

Other extensive reviews of the literature have been published in which different analytical approaches have been used. Dore et al., (1997), examined published reports from 1992 to 1996 in which HCV RNA data were available. They found 903 infants born to mothers with viremia, indicated by detectable HCV RNA, in the literature and determined the overall rate of mother-to-infant transmission of HCV to be 6.2% (95% CI: 4.6-7.8%) and essentially 0% in mothers without demonstrated viremia. They further categorized the rate of transmission as 15.8% (95% CI: 11.8-19.8%) for mothers co-infected with HIV and 1.9% (95% CI: 1.2-2.6%) for mothers known to be negative for HIV or of indeterminate status for HIV. Thomas et al., (1998), reviewed 116 published reports and other unpublished reports. They applied strict diagnostic criteria and excluded studies with brief follow-up of the at-risk infants; they identified 976 infants in 28 studies who could be evaluated. They found that the rate of mother-to-infant transmission of HCV was less than 10% in HIV-negative women in most unselected populations, higher in the presence of HIV co-infection, and almost always associated with HCV viremia in the mother, although they could not exclude technical factors leading to viral RNA degradation before analysis.

Findings from the most recent prospective studies have been similar. In a study from Ireland of 314 infants born to 296 anti-HCV-positive women, (Healy et al., 2001) the rate of mother-to-infant transmission was 3.5%
(minimum rate) to 6.4% (based on observed cases). No significant differences were found with spontaneous rupture of membranes, duration of membrane rupture, vaginal delivery or cesarean section, or evident fetal distress. Infants tended to be small for gestational age, but this could not be attributed solely to maternal chronic hepatitis C. In a study of 2,447 HIV-negative pregnant women from Italy (Ceci et al., 2001). 78 women were identified as anti-HCV positive and these mother-child pairs were monitored for 2 years; 60 women were found to be HCV-RNA positive. Eight infants were identified as infected with HCV. Thus, the mother-to-infant transmission rate was 13.3%. At 2 years old, however, only 2 infants were still positive for HCV-RNA, and therefore, the overall mother-to-infant transmission rate was stated to be 3.3%. Mother-to-infant transmission correlated with high maternal viral levels. In a large retrospective analysis of prospectively collected data on 1,655 mother-child pairs from 24 centers in Europe (specifically Italy, Spain, Germany, Ireland, Scotland, Belgium, and Sweden), 9.2% of evaluable infants were infected with HCV (EPHCV, 2001). Mother-to-infant transmission of HCV was more likely to occur if the mother was co-infected with HIV, but mode of delivery and breast-feeding did not affect HCV transmission in women with only chronic hepatitis C. In a recent prospective multicenter study from Italy, the rate of mother-to-infant HCV transmission was examined in 1,372 consecutive, unselected mother-infant pairs. The rate was 7.1% (95% CI: 2.2 - 7.2%). All but 1 infant was born to women with detectable serum HCV-RNA, and in this study maternal injection drug use, not maternal HIV positivity, was most closely associated with HCV transmission (Resti et al., 2002). Rate of HCV transmission was not statistically different in women having cesarean section as opposed to vaginal delivery; however, the rate of elective cesarean in women not co-infected with HIV was very low.
Whether the risk of transmission of HCV is independent for each pregnancy has been examined formally in one study (Resti et al., 2000). No increased risk for mother-to-infant transmission of HCV was found in subsequent pregnancies.

2.22 VIRAL LEVELS

Numerous, but by no means all, (Resti et al., 1998; Conte et al., 2000) studies indicate that the higher the concentration of serum HCV RNA the more likely mother-to-infant transmission (Lin et al., 1994; Ohto et al., 1994; Moriya et al., 1995; Matsubara et al., 1995; Okamoto et al., 2000; Ceci et al., 2001) In one study, a high viral level was defined as at least 2.5x10^6 viral RNA copies per mL (Okamoto et al, 2000) but in general, studies showing a correlation of HCV transmission with maternal viral load exhibited the effect at 10^5 to 10^6 copies per mL. Differences in when during the course of pregnancy maternal viral levels are tested may explain some of the conflicting observations on this point.

2.23 HIV CO-INFECTION

Co-infection with HIV has consistently been associated with greater likelihood of transmitting HCV to the newborn infant (Ohto et al., 1994; Paccagnini et al., 1995; Zanetti et al., 1995; Tovo et al., 1997; Granovsky et al., 1998; Gibb et al., 2000; EPHCV 2001). There was no increased rate of transmission of HCV to infants of HIV co-infected women in the large cohort reported by Conte et al., (2000), but all of these mothers had received anti-retroviral treatment during pregnancy an observation which suggests that
HIV infection in HIV/HCV co-infected women should be treated aggressively to reduce the risk of mother-to-infant HCV infection.

2.24 HCV GENOTYPE

Maternal-infant transmission occurs with all known genotypes. To date, no obvious correlation between HCV genotype and rate of mother-to-infant transmission of HCV has been shown (Zuccotti et al., 1995; Zanetti et al., 1999).

2.25 DISEASE ACTIVITY

There have been few observations relating disease activity in the mother and likelihood of transmission of HCV infection to the infant. In general, hepatic inflammation appears to decrease during pregnancy, as shown by a decrease in serum ALT levels in the third trimester. A single report has shown that mother-to-infant transmission correlates with peripheral blood mononuclear cell infection by HCV (Azzari et al., 2000).

2.26 INVASION PROCEDURES

Some evidence is available to show that amniocentesis is a potential risk for spreading infection to the infant (Minola et al., 2001). However, HCV was found in amniotic fluid from only 1 of 16 viremic patients studied prospectively in the fourth month of pregnancy (Delamare et al., 1999). On reasonable, common sense grounds, the use of fetal blood monitoring via scalp vein catheter has been discouraged in the presence of maternal HCV infection, and some obstetrical units prohibit this intervention altogether.
However, firm data to indicate that fetal monitoring places the infant at risk for acquiring HCV infection are difficult to ascertain. One recent large study done by European Paediatric Hepatitis C Virus Network (EPHCV, 2001) found 11.8% of 93 infants who had fetal scalp vein blood monitoring during delivery developed mother-to-infant HCV infection, whereas 9.2% of 631 infants not monitored developed HCV infection, but it was not clear whether the monitored infants had any other known risk factors for acquiring infection.

2.27 COMPLICATION OF DELIVERY

Women with chronic hepatitis C may be at risk for complications of pregnancy and delivery for reasons independent of the HCV infection. Some observations suggest that prolonged rupture of the membranes (i.e., for more than 6 hours) is associated with a higher rate of mother-to-infant transmission of HCV (Spencer et al., 1997) but other studies have not shown this association (Healy et al., 2001).

2.28 TYPE OF DELIVERY

Some studies suggest that mother-to-infant transmission of HCV is more likely to occur with vaginal delivery (Paccagnini et al., 1995; Granovsky et al., 1998; Okamoto et al., 2000; Gibb et al., 2000). Other studies do not confirm this finding (Ohto et al., 1994; Resti et al., 1998; Conte et al., 2000; Hillenmanns et al., 2000). Moreover, in some of the studies indicating increased risk with vaginal delivery, the difference fell short of statistical significance, including a large European study where mode of delivery was known for 1,400 infants (EPHCV, 2001) Elective cesarean section may
confer some protection, but emergency cesarean section does not (Gibb et al., 2000). In 11 studies with at least 10 mother-infant pairs for evaluation, the rates of mother-to-infant HCV were similar for vaginal delivery and cesarean section. The weighted rate was 4.3% for vaginal delivery and 3.0% for cesarean section (Yeung et al., 2001). Currently available data, therefore, do not argue cogently for the routine use of cesarean section for women with chronic hepatitis C unless there is HIV co-infection.

2.29 TIME/MODE OF MOTHER-TO-INFANT TRANSMISSION OF HCV INFECTION

When or how mother-to-infant transmission of HCV occurs remains unknown. Hepatitis C virus RNA is occasionally detectable in amniotic fluid, (Delamare et al., 1999; Minola et al., 2001) and although this finding raises the possibility of in utero infection, it does not equate with in utero infection. Cord blood positivity for HCV-RNA is extremely difficult to interpret as an indication of in utero infection because minute contamination with maternal blood cannot be excluded. In one study, cord blood positivity for HCV-RNA was not predictive of subsequent infection in the infant (conte et al., 2000). The possible effect of elective cesarean section to diminish risk of mother-to-infant transmission argues for infection at or around the time of vaginal delivery (Okamoto et al., 2000; Gibb et al., 2000). Finding HCV-RNA in the serum of the newborn infant in the first few days of life is consistent with infection occurring either in utero at the time of delivery. However, finding HCV-RNA in the serum of the newborn infant within 24 hours of birth would likely favor in utero infection. There could, of course, be more than one time or mode of infection in different patients. In the recent European multicenter study, 11 of 203 children in whom HCV-RNA testing was obtained in the first
3 days of life were subsequently shown to have mother-to-infant transmission of hepatitis C; however, only 3 of the 11 had HCV-RNA detectable within the first 3 days of life (EPHCV, 2001).

Patterns of HCV quasispecies expression in mother-to-infant HCV infection provide some clues to the time/mode of infection. Thus, infants infected with HCV appear to have one or very few quasispecies of HCV initially (Weiner et al., 1993; Kudo et al., 1997; Manzin et al., 2000; Sitia et al., 2001). The major quasispecies infecting the infant are not necessarily the dominant form in the mother. The mechanism for this apparent selectivity is not known, but it is possible that interferons generated in the placenta may play a role (Zdravkovic et al., 1997).

2.30 BREAST-FEEDING AND TRANSMISSION OF HEPATITIS C

Hepatitis C virus can be detected in breast milk or colostrum (Bernard et al., 1998; Ruiz-Extremera et al., 2000). Nevertheless, breast-feeding is generally not considered to be a risk factor for mother-to-infant transmission of HCV (Lin et al., 1995; Spencer et al., 1997; Polywka et al., 1999; Conte et al., 2001). In published studies, the rate of transmission is nearly identical in breast or bottle-fed infants (Yeung et al., 2001). Whether these studies are adequate is open to question because duration and exclusivity of breast-feeding are not routinely described in detail. The safety of breast-feeding operates on the assumption that traumatized, cracked, or bleeding nipples are not present. A single study from United Arab Emirates attributed disease acquisition (characterized as acute hepatitis C) in 3 infants, born by cesarean section, to exposure by breast-feeding; however the mothers of these children had unusually severe clinical disease with elevations in serum
ALT and bilirubin levels in the immediate postpartum period (Kumar et al., 1998). Although these observations may not be easily generalized to others, breast-feeding during a postpartum flare of hepatitis may pose a risk of HCV transmission to the infant.

2.31 ISSUES RELATING TO ANTI-VIRAL FOR CHRONIC HEPATITIS C IN PREGNANCY

Treatment with the most efficacious therapy (alpha interferon combined with ribavirin) cannot be used during pregnancy or immediately before pregnancy, primarily because ribavirin poses an important risk of teratogenicity (Kilham et al., 1977). Scattered case reports have been published suggesting that the potential risk associated with ribavirin is overestimated, but these data are too limited to be convincing (Mishkin et al., 2001; Hegenbarth et al., 2001). There is some experience with administration of alpha interferon to pregnant women who have idiopathic thrombocytopenia, chronic myelogenous leukemia, or other hematologic malignancies. No adverse outcomes were found in the babies except for transient thrombocytopenia in one (Mubarak et al., 2002). Alpha interferon is contraindicated in infants below 2 years of age because of severe neurotoxicity (Barlow et al., 1998; Dubois et al., 1999).

2.32 OUTCOME OF MOTHER TO INFANT HEPATITIS C IN AFFECTED INFANTS

The outcome of mother to infant hepatitis C requires clarification. Some infants may have transient viremia without real infection. Other infants may have acute, self-limited infection that is clinically in apparent (very early
spontaneous resolution). The data for very early spontaneous resolution of mother-to-infant hepatitis C are compelling in those children who developed biochemical evidence of hepatic inflammation at the time of hepatitis C viremia (Ni et al., 1994; Bortolotti et al., 1997; Sasaki et al., 1997; Spencer et al., 1997; Xiong et al., 1998). In one study, three fourths of the infants identified with mother to infant hepatitis C cleared the HCV RNA by 2 years of age (Ceci et al., 2001). In another study, all infants with mother to infant hepatitis C lost detectable serum HCV RNA by 6 months of age (Ketzeni-Gilad et al., 2000). Thus, outcome of mother to infant transmission of HCV is usually considered in terms of evolution to chronic hepatitis C, with later spontaneous clearance of HCV infection or progressive chronic liver disease. Whether children are more likely to clear chronic HCV infection than adults and whether transfusion-associated chronic hepatitis in children runs a different clinical course from chronic hepatitis C acquired by mother to infant transmission remain unanswered questions.

2.33 HCV TRANSMISSION IN INDIA

Understanding of the epidemiology of HCV in India would need assessment of prevalence of anti-HCV antibodies (measure of HCV exposure) and if possible, the proportion of viremic individuals in age-stratified general population, high-risk groups as well as in patients suffering from various liver disorders. However community based age-stratified surveys has been limited from India so far. One such study from Western India have reported a very low prevalence of anti-HCV antibodies among 295 children and 430 pregnant women screened (Arankalle et al., 1995). HCV infection in voluntary blood donors and general population is reported to be ranging from 1.85% (Delhi) to 2.5%. (Hyderabad) (Panigrahi et al.,
1997; Khaja et al., 2002). Surprisingly 15.9% anti-HCV positivity was recently reported from Mumbai, among voluntary blood donors, which requires immediate surveys for validation (Gosavi et al., 1997). Recent studies from Northern India have reported 5.2% positivity for anti-HCV among North Indian urban children (Arora et al., 1999). Among the pregnant women screened in the same area, the positivity was found to be 1.33% (Irshad et al., 1998) However no data is available on the transmission of HCV from infected mothers to their infants.

Though majority of the Indian population resides in rural areas, studies dealing with exposure of such population to HCV are scanty. Of the 1054 serum samples collected in 1995 and representing 602 children (<15 years of age) and 452 adults (>15 years of age), only one was reported as anti-HCV positive (Chadha et al., 1999).

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. There seems to be immediate need for conducting multicentric studies dealing with certain important issues such as:

1. To define healthy HCV carrier state, if at all it exists.
2. Risk factor analysis for acquiring HCV infection and leading to chronic HCV infection.
3. Prospective follow-up of subjects with chronic HCV infection.
4. To screen pregnant women and children born to them if the mother is viremic.