Discussion
6. DISCUSSION

6.1 LIVER DISEASE GROUP

6.1.1 SPORADIC ACUTE VIRAL HEPATITIS

Studies have shown that the vast majority of the Indian population are exposed to HAV early in childhood in the form of icteric and anicteric infections (Tandon et al, 1984; Roy et al, 1987). Therefore, since conversion to HAV apparently confers life-long immunity, clinical hepatitis A is relatively rare in the adult Indian population. In the present study, as the study group included only adults, infection due to HAV was seen in only 3% of the total cases.

Hepatitis B happens to be the major aetiological agent of sporadic AVH in India as reported among cases from different parts of the country (Datta et al, 1987). The HBV positivity in this study was reported to be between 34.6% to 51% (average 37.8%). Similarly, higher rates of HBV infection were reported among sporadic AVH cases from the West (Bamber et al, 1983; Francis et al, 1984). In the present study HBV infection was responsible for 47.4% of the cases.

A large number of cases, negative for markers of HAV and HBV, were found in sporadic AVH. These cases, termed as Non-A, Non-B hepatitis (NANBH) were found to constitute 44% and 61.5% of sporadic AVH in adults
in North and South India respectively (Tandon et al., 1984; Dutta et al., 1987). The NANBH constitutes 9-18% of the sporadic AVH cases among developed nations (Ketiladze et al., 1979; Papaevangelou et al., 1979; Silva et al., 1983; Greenfield et al., 1984). In the present study, NANBH was found in 44.1% of the cases which is comparable to that of the earlier Indian reports. However when these cases were further analysed for markers of HCV and HEV, the Non A-E group came down to 19.8% only.

HCV positivity of 1.7% is in agreement with the previous study done in India by Khuroo et al.(1993). This may probably be due to the anicteric course of acute HCV infection which more often slips onto chronicity.

HEV has been the major aetiological agent in sporadic NANBH cases in India. HEV positivity of 27.9% and 41% have been reported among sporadic AVH cases from Kashmir (Khuroo et al., 1994) and Delhi (Acharya et al., 1995) respectively. In the present study HEV has been responsible for 22.5% of the total cases and 51.1% of the NANBH cases which is very similar to earlier reports.

6.1.2 TRANSFUSION-ASSOCIATED ACUTE VIRAL HEPATITIS

Viral hepatitis is believed to be almost as old as recorded history, but the first generally accepted reference to parenterally-transmitted hepatitis is that of an outbreak of "jaundice" in 1885 that affected approximately 15% of a group of 1289 Bremen shipyard workers (Lurman, 1885). A dramatic epidemic in 1942 of icteric hepatitis involving 50,000 US servicemen
represented the final proof of the existence of parenterally transmissible hepatitis (Sawyer et al., 1943). The outbreak was proven to be of HBV origin through a serological analysis conducted 40 years later (Seeff et al., 1987). Even after the development of diagnostic assays for HAV and HBV and exclusion of HBV positive donors in early 1970's, there were cases of PTH, which were termed as parenteral type of NANBH. In 1989, after the discovery, cloning, sequencing and development of an assay for HCV, it was found that 90% of the PTH was due to HCV (Choo et al., 1989).

The prevalence of anti-HCV was found to be very high in patients transfused with blood or blood products, with rates of 60-80 % in those with chronic transfusion associated NANBH, 50 % in patients with acute resolving NANBH and 60-90 % in haemophiliacs receiving commercial clotting factor concentrates (Brettler et al., 1990; Allain et al., 1991; Blanchette et al., 1991). In the present study, 78 patients with Post-transfusion AVH were analysed for the hepatitis viral markers and was found that HBV was responsible for majority(62%) of the cases and HCV was found positive in 20(25.6%) of the total cases and 69% of the NANBH cases. The high HCV positivity is very much foreseen, as testing for anti-HCV is still not mandatory in India, but the high HBV positivity among the PTH cases is alarming, as screening for HBsAg is mandatory for blood donors in India. The successful implementation of screening for HBsAg and anti-HCV has brought down the number of PTH cases remarkably in many countries, similar implementation programmes have to be followed in India if the PTH cases have to be reduced.
Another significant finding in the present study was comparing transfusion as a risk factor for the transmission of HBV and HCV. Transfusion was found to be a significant risk factor for the transmission of HCV. This conclusion was based on the results obtained in the study that in sporadic AVH cases, where there was no history of transfusion in any of the cases, the HCV percentage was minimal, comparable to that found among healthy population (p > 0.05). Whereas, in the 78 AVH patients with a history of transfusion, it was seen that HCV positivity rose to 25.6% (p < 0.001). However, the HBV positivity difference in sporadic (47.5%) and transfusion-associated AVH (62%) cases was not statistically significant (p > 0.05).

6.1.3 FULMINANT AND SUBACUTE HEPATIC FAILURE

Most reports on fulminant hepatic failure have been predominantly from the West (O'Grady et al., 1993; Muto et al., 1993; Bernau et al., 1986). The leading agents of fulminant hepatic failure in India are hepatitis viruses (99%) (Tandon et al., 1986) unlike in the West, where the major cause of fulminant hepatic failure are drugs, HAV and HBV (Bernau et al., 1986; Toghill, 1969).

In India, HBV has been reported to be responsible for 25-35% of the fulminant hepatic failure cases and the majority of the cases are due to NANBH viruses (Raju et al., 1989; Irshad and Acharya, 1994). After the identification of the aetiological agents of NANBH, HEV has been implicated as the major cause of fulminant hepatic failure with significant mortality rates in pregnant women (Khuroo et al., 1995; Nanda et al., 1994; Acharya et al., 1996).
The role of HCV in fulminant hepatic failure has elicited a lot of controversial results. HCV was found in 45% of the FHF cases in a study by Irshad and Acharya (1994). But the HCV positivity was found in only 7.9% of the FHF cases in a study by Arankalle et al (1995). In a later study by Acharya et al (1996), HCV was found as a co-infecting or superinfecting agent rather than as a single infecting agent. It was indicated that either HCV infection caused the disease or a HCV carrier state made these patients more susceptible to another hepatotrophic viral infection resulting in severe liver injury, as most of the FHF cases with HEV-RNA or any other acute viral marker had HCV-RNA in them.

The picture in the Western countries is entirely different with HBV being responsible for 40-60% of the FHF cases (Trepo et al., 1976; Mathiesen et al., 1980), NANBH not being responsible for a significant proportion of cases and HCV and HEV not being incriminated as the aetiological agents of fulminant hepatic failure (Sallie et al., 1991; Wright, 1993). But the scenario in Japan is entirely different from these observations. In a study by Yanagi et al (1991) it was found that HCV was the major causative agent for NANBH cases of fulminant hepatic failure in Japan.

Similarly, the role of HCV in subacute hepatic failure (SAHF) is rare. There have been very few reports on the role of HCV in SAHF, especially in India. In a study by Irshad and Acharya (1994), it was seen that HCV was positive in 44.6% of the 65 cases screened and HBV was positive in only 23% of them. In another study by Tandon et al (1994), HCV was positive in 15 %
of the SAHF cases. Whereas in the present study the HBV positivity was found to be 56.25% and none of the cases were found to be positive for HCV. No definite conclusion could be arrived regarding the role of HCV in SAHF, as the sample size was small. One possible explanation for the absence of HCV among SAHF cases could be that none of the 16 cases screened had received blood transfusions.

6.1.4 CHRONIC LIVER DISEASE

Virological status

One common problem in chronic liver diseases is the high prevalence of hepatitis B virus (HBV), which is on top of the list of causes of chronic hepatitis, liver cirrhosis and liver cancer (Suzuki and Woodfield, 1994). HCV ranks significantly behind HBV in the pathogenesis of CLD in the U.S., former USSR and most Asian countries, except Japan where HCV is the leading cause (Suzuki, 1994). The difference in positivity reflects the carrier rates of these two viruses in the general population of different countries.

Highest HBV positivity among CLD was seen in China with 74% positivity for HBV and 13% positivity for HCV. Among CLDs the lowest HBV positivity (26%) was seen in Indonesia with a HCV positivity of 16%. Most of the countries had a HBV positivity between 35-65% and a HCV positivity of 12-35%. In the present study, HBV positivity was recorded in 39% of the cases and HCV positivity was seen in 17.6% of the cases, which is very similar to that observed in the above reports. The NBNC percentage among the CLDs had been reported to be between 3-35%. However, in the present study it was
seen that the NBNC was recorded in 48.6% of the cases, which is high and comparable only to that found in Indonesia with 60% NBNC cases (Sulaiman, 1994). Even the previous Indian reports have implicated HBV as the major etiologic agent of CLD followed by HCV (Ramesh et al, 1992; Sumathy et al, 1992; Amarpurkar et al, 1994; Nanda et al, 1994, Mehta et al, 1994; Tandon et al, 1994). The reported HBV positivity in these studies was between 40-72% and HCV positivity was recorded in 16-26% of the CLD cases. However, in one study (Irshad and Acharya, 1994), a high HCV positivity of 48.5% was observed, of course the patient population in this study consisted of only CAH cases.

Even among the HCC cases, HBV has been implicated in the majority of the cases. Most of the countries have reported a HBV positivity of 32-80% among HCC (Suzuki, 1994; Ramesh et al, 1992; Tandon et al, 1994; Shrestha, 1994; Tao et al, 1994; Liaw, 1994), the highest being Thailand with 92% HBV positivity among the HCC cases (Chainuvati et al, 1994). HCV prevalence had been recorded in 3-17% of the HCC cases in these studies. In the Indian scenario, Ramesh et al (1992) had reported a HBV and HCV seroprevalence of 28% and 15% respectively among the HCC cases. The present study shows that HBV was the aetiological agent in 67% of the cases and HCV was seen in 22.7% of the HCC cases. The HCV positivity is low, both among CLDs and HCCs in the present study when compared to studies from Japan, Spain and Italy where the frequency of anti-HCV is more than 80% in CLD and HCC patients (Colombo et al, 1989; Suzuki, 1994).
Significance of PCR

The importance of PCR is in differentiating the viraemic from the non-viraemic phase and also to identify mutants. In the present study, a high HBV-DNA positivity (97.2%) was recorded in the HBeAg positive cases and a low HBV-DNA positivity (57.3%) in the HBeAg negative cases. This is in total agreement with the established literature that HBeAg is a marker of viral replication and when it is positive, naturally the HBV-DNA is also positive (Scott et al, 1990; Pontisso et al, 1992; Ljunggren et al, 1993).

Not all cases suffering from CLD can be diagnosed by the conventional enzyme immunoassay systems (EIA), there are certain cases which are missed by these assays. However, the PCR assay is sensitive enough to pick up these EIA-negative cases as positive ones, and project the true etiological picture (Thiers et al, 1988; Paterlini et al, 1990). In the present study, of the 153 HBsAg negative cases tested for HBV-DNA, 16 (10.5%) were positive by the PCR. The HBV positivity rose from 39.6% to 42.2% when these 16 cases were added to the 245 HBsAg positive cases. Even in the diagnosis of HCV infection, not all HCV-infected cases were picked up by the EIAs as seen in earlier reports. The anti-HCV negative cases and a higher number of RIBA indeterminates were shown to be HCV-RNA positive by the PCR (Lavanchy et al, 1994; Pawlotsky et al, 1994; Goffin et al, 1994). Even in the present study, the HCV positivity by anti-HCV reactivity alone was 14.8% but when the RIBA indeterminate and anti-HCV negative cases were subjected to the RT-PCR, there were additional HCV positives and the HCV positivity rose to 18.2%.
Age and Gender

The correlation of sex and age with the occurrence of hepatitis viral markers has also been demonstrated in the present study. There was a male preponderance in both HBV-positive and HCV-positive groups. Similar observations have been reported previously by Resnick and Koff (1993). The age of the patients was significantly low (p<0.001) at the earliest phase of the CLD (CHP) and increased steadily till it reached HCC. Even among the HCC group, there was a definite association between viral positivity and the age of the patient. As reported by Saito et al (1990), even in the present study HCV positive individuals were significantly older than their HBV positive counterparts(p<0.05). This could be due to the fact that infection with HBV is acquired early in life than HCV.

Transfusion as Risk factor

The significance of transfusion as a factor for the transmission of hepatitis viruses is common knowledge today. However, it was seen that there was no significant difference in HBV positivity in the transfusion-associated CLD group and the non-transfusion-associated CLD. But there was a significant difference in the HCV positivity between the transfusion-associated CLD group and the non-transfusion-associated CLD. This finding suggests that besides transfusion there might be various other modes of transmission for HBV, whereas for HCV, transfusion/ exposure to infected blood is the major cause, with other lesser known routes accounting for a minor percentage of cases only.
6.2 HIGH-RISK GROUPS

6.2.1 Chronic Renal Failure (CRF) Cases

Hepatitis C virus infection is very common among maintenance haemodialysis patients throughout the world. The prevalence seems to reflect the quality of medical practice being offered in the country as does the anti-HCV prevalence among the general population there. HBV infection in patients and hospital staff at dialysis units was once rampant, but it has been steadily reduced in incidence with the separation of patients and adherence to other preventive measures including vaccination against hepatitis B. With the availability of diagnostic systems for HCV, similar measures are being taken up for the prevention of HCV infection in dialysis units. HCV attracts a wider attention than HBV because of its higher chronicity rates and non-availability of vaccine.

The prevalence of HBV among dialysis patients and hospital staff is well documented. 41% of the haemodialysis centers reported sporadic cases of hepatitis infections in a survey conducted in the USA by the Centers for Disease Control (CDC) during 1967-68. Besides sporadic infection, a number of outbreaks have been reported at dialysis centers (Almeida et al, 1971; Garibaldi et al, 1972; Hennekens, 1994). With rigorous enforcement of the preventive measures for nosocomial infections, the incidence of HBsAg among dialysis patients has been remarkably reduced in the United States and elsewhere. According to Alter et al (1990), infection incidence was 3.0% among patients in 1976, 1.0% in 1980, 0.5% in 1983 it was down to 0.2% in 1988. The
prevalence rate for HBsAg also came down from 7.8% among patients in 1976 to 2.4% in 1983. In the present study, HBV positivity was found to be 35.1%, which was high and very similar to that found in some of the developed and developing countries. This could indicate a serious lacunae in the diagnostic systems adopted for screening and/or unsuccessful implementation of mandatory screening.

In the present study, HCV positivity was seen in 36.9%, of the cases, which is again high and similar to reports from other countries. The high number of HCV positive cases was as anticipated, as the screening for anti-HCV is still not mandatory in blood banks in India. The HCV positivity was found to be between 5.5% and 38.6% by the first generation ELISA in previous reports from other countries (Roggendorf et al, 1989; Tamura et al, 1990; Huang et al, 1993). HCV positivity was found to be between 18% and 74.2% when tested by the second generation ELISA in some countries (Sakamoto et al, 1993; Viola et al, 1993; Pujol et al, 1996; Luengrojanakul et al, 1994).

Oguchi et al (1993) compared 607 patients on dialysis and 704 blood donors of comparable ages; anti-HCV was positive in 17.1%, anti-HBs in 18.1% and anti-HBc 36.7% in the former, and in the later, these figures were 0.9%, 8.9% and 16.6% respectively, the differences being highly significant. Even in the present study when the HCV positivity was compared between the dialysis patients and blood donors (33.3% vs 0.86%) the difference in the positivity was highly significant (p<0.001). There have been reports that there is an increase
in virus transmission if there is an increase in transfusion units or duration of dialysis. In a study by Irie et al (1994) it was shown that anti-HCV positivity rose from 4.6% in cases before dialysis to 50% after being exposed to dialysis for 10 years. In a similar study by Tamura et al(1992), it was demonstrated that there was a significant increase in HCV positivity between patients undergoing haemodialysis for less than 3 years and in patients undergoing haemodialysis for more than 3 years, regardless of transfusion. In the present study we observed a significant increase in HCV positivity with the increase in transfusions or haemodialysis, which was very similar to previous reports. However, this trend of increasing positivity associated with increase in transfusion units or dialysis was not seen in the case of HBV (p>0.05). This may be because of the fact that only HBsAg screening was done in these cases. If anti-HBc and anti-HBs screening was also conducted, a picture would have emerged that would truely represent the relative role of HBV and HCV.

The transmission of HBV and HCV in dialysis units is best diagnosed by testing for the viral markers itself than looking for clinical symptoms of acute infection or elevated ALT levels. The present study has shown that, of the 97 viral positive cases only 19 had AVH and only 23 had elevated ALT levels. This picture is very similar to that reported earlier by Silini et al (1993) and Caramello et al(1993). The transmission of HBV and HCV infection in the two follow up cases clearly demonstrates and further strengthens the previous findings that haemodialysis and/or transfusion/transplantation are high risk practices for hepatitis viral transmission (Tamura et al, 1992; Irie et al, 1994).
6.2.2 Intravenous Drug Users (IVDU)

Individuals sharing syringes or needles for IVDU are at high risk for infection by parenterally-transmitted infectious agents. Consequently, the prevalence of anti-HCV in IVDUs has been reported to be high. The reported prevalence range from 48-80% in a number of studies carried out in developed countries (Esteban et al, 1989; Mortimer et al, 1989; Roggendorf et al, 1989, van der Poel et al, 1991). Alter MJ et al (1990b) reported a HCV prevalence of 55% in IVDUs in the USA when tested within 6 weeks of the onset of illness; however, this figure rose to 94% when these individuals were monitored for extended periods of up to 4 years. In the present pilot study of IVDUs among Indian population, HCV positivity was found to be 63.8%, which is high and very similar to reports from abroad.

Interestingly, a lesser percentage of cases (24.8%) were found positive for HBV in the present study. This had a further consequence on the HDV positivity (4.5% of the HBV positive cases and 1.1% of the total cases), which again was low. This picture was completely different from that found in Europe and the USA where HDV infection is relatively high among IVDUs. In a multicentric study from Europe, between 31% and 75% of HBsAg-positive drug addicts had markers of HDV infection (Raimondo et al, 1982). Similarly, among a group of 372 addicts in New York, 80 (22%) had anti-HDV (Kreek et al, 1990). The reversal in positivity rate of HBV and HCV in the present study is intriguing as HBV is many more times infectious than the HCV and so are the chances of getting infected (Houghton et al, 1991). One possible explanation for
the low HBV positivity could be that these cases could have been already infected with HBV and seroconverted naturally. Hence, anti-HBc anti-HBs testing could really indicate the prevalence of HBV among such population.

Though viral positivity among IVDUs in India is high, the percentage of cases having an acute infection among them is very low. Only 52.2% of HBV positive cases and 21.2% of HCV positive cases had a history of acute infection. Almost all of the IVDUs, except one, were asymptomatic. This observation again is very different from that reported from the West, where majority of the IVDUs with viral positivity had clinical symptoms (Alter et al., 1990). In the present study, ALT levels were elevated in only 29.5% of HBV and 24.7% of HCV cases. Overall, the ALT levels were within the normal range among the IVDUs.

6.2.3 Health Care Workers

Viral hepatitis has been recognised as an occupational risk in certain occupations and on the other hand, individuals with hepatitis infection pose a risk of transmission to others in the course of their work. The most important means of transmission of viral hepatitis in the occupational setting is by inoculation of infected blood, either by stab injuries with blood-contaminated needles (so-called needle stick injuries) or by cuts with scalpels or other sharp instruments contaminated with blood (sharp injuries) (Favero et al., 1990).
The prevalence of hepatitis B markers among a group of emergency physicians was found to be five times higher than in the general population in the USA (Iserson and Criss, 1985). It has also been estimated by the CDC (1989) that 12,000 health care workers whose jobs entail exposure to blood become infected with hepatitis B each year. Even in countries where hepatitis B infection is endemic, there is evidence of additional occupational risk among health care workers. In Japan, a seroprevalence study found that over a third of hospital workers had evidence of previous hepatitis B infection, about the same prevalence as a group of healthy controls, but that nurses and surgeons had significantly higher seroprevalence than other staff or the controls (Kashiwagi et al, 1985). The present study reports a similar situation where in the HBV positivity is 6.4% among the total number of health care workers screened, and was found to be positive among 8.9% of Doctors and Nurses, which was high.

The carrier state among health care workers is of prime concern because they could transmit the virus to patients resulting in outbreaks (Gerlich and Thomassen, 1982; Mijch et al, 1987) and in the present study it was observed that HBV-DNA positivity or infectivity was high.

The mode of occupational transmission of hepatitis C appear to be similar to those of hepatitis B. Several workers have reported on the prevalence of hepatitis C antibodies or the frequency of non-A,non-B hepatitis among occupational groups exposed to blood. Alter et al (1990) reported that 2% of patients with non-A,non-B hepatitis in their sentinel counties
surveillance in the USA had occupational exposure to blood. Mortimer et al (1989) found that none of the 100 hospital staff reporting inoculation injuries were anti-HCV positive. In another study, although only 0.58% of 1033 hospital employees had antibodies to hepatitis C, this prevalence was found to be significantly greater than the 0.24% HCV positivity among 2113 blood donor controls (Jochen, 1992). Klein et al (1991) found antibodies to hepatitis among 2% of 456 dentists in New York compared with 0.1% of 723 controls; among a small group of oral surgeons the prevalence was 9%. In the present study HCV positivity was found to be 5% among the health care workers, when compared to 0.89 % HCV positivity among the blood donor population. Though the study group was small, this study certainly elicits the risk involved in a health care setting for the transmission of hepatitis viruses.

6.3 BASELINE DATA

6.3.1 Healthy population

Transfusion-transmitted infections are largely preventable if the appropriate screening programmes are designed and implemented. The use of screening assays themselves should form only one part of an overall strategy for ensuring the safety of transfused blood and its derivatives. Donors infected by HCV, and therefore potentially at risk of transmitting the virus, are initially identified by the presence of antibodies to the virus in the serum.

The seroprevalence of HCV has been divided into three categories (based on the HCV positivity among the healthy population)-i) low (with HCV positivity in < 0.5% of the population), ii) medium (with 0.5-2.0% HCV
positivity) and iii) high (> 2% HCV positivity). The HCV prevalence rates are low in Northern Europe and the USA, medium in Southern Europe and some Asian countries and high in certain other Asian countries (Casselman and Alt, 1996). The HCV seroprevalence is highest among the African population. However, some isolated studies have reported a slight deviation, either lower or higher, in HCV prevalence in the above reported geographical areas (Frommel et al, 1993; Bassily et al, 1995).

The present study indicates that Southern India (Tamilnadu) falls in the medium range for HCV seroprevalence. Interestingly indeed, the HCV prevalence in India falls in all the three categories, i.e., high, medium and low, based on the geographical location. The Northern part of the country has reported a high HCV seroprevalence between 1.5% and 2.7% (Irshad et al, 1995; Panda et al, 1996). In fact, the 2.7% HCV seroprevalence reported by Panda et al (1996) is much higher than that reported from other Asian countries (Tamura et al, 1992). The Western part of the country has reported a low HCV seroprevalence of 0.12% (Arankalle et al, 1995), though the HCV positivity is much higher in the professional donors than the voluntary donors. The present study indicates that Southern India falls in the medium range, though a previous study (Abraham and John, 1995) had reported a 0.48% HCV seroprevalence in Southern India (Vellore).

Of the 9 anti-HCV positive cases, 6 (66.6%) were found to be positive for HCV-RNA. The HCV-RNA status among the blood donors is very important because current data suggests that donors with circulating HCV-RNA are more
likely to transmit HCV through transfusion than those possessing anti-HCV in the absence of RNA (Farci et al., 1991).

6.3.2 Risk factor analysis for HCV transmission

The transmission of HCV may conveniently be considered in relation to the specific route of infection and the particular groups of individuals at risk of infection. Although the mechanism of transmission of hepatitis C virus is similar to that of HBV, it is clear that there are some significant differences. For example, in the present study among the IVDUs and the CRF cases, the HCV positivity was higher than the HBV. Current data suggests that in developed countries the source of HCV infection in infected individuals can be grouped as follows: in approximately 38.7% of patients the source of transmission is undefined; 20.6% of patients have a history of intravenous drug abuse; 34.4% of patients have a history of transfusion; 3.8% heterosexual contact, 0.6% tattoo and 2% are health care workers (Alter et al., 1989; Insight, 1992; Zeuzem et al., 1996).

In the present study, intravenous drug abuse and transfusion are the risk factors in 35.2% and 37% of the HCV positive cases respectively. This is in total agreement with the previous reports where it has been recorded that, among IVDUs the HCV positivity is between 48-80% in developed countries (Esteban et al., 1989; Mortimer et al., 1989; Roggendorf et al., 1989). Likewise, HCV contributes to 80-90% of post-transfusion NANBH with transfusion as the risk factor (Alter et al., 1991c). Hospitalisation was the only risk factor encountered in 8% of the HCV positive cases in the present study.
Haemodialysis and health care employment has been found as a risk factor in a minor percentage of cases (2.8% and 0.9% respectively) in the present study which is very similar to previous reports (Alter et al., 1990).

However, what is of prime concern is the fact that 13% of the HCV positive cases have no-attributable risk factor. This finding is very similar to reports by Alter et al. (1990) and Kelen et al. (1993) that 10-40% of the HCV positive cases have no identifiable risk factor. According to them, the no-risk-factor infections are as a result of underreporting of illicit drug use and other risk behaviour like the sexual practices. The HCV positivity due to sexual transmission was found to be in 9% of the cases in previous reports (Alter, 1990). The present study did not deal with the sexual and vertical mode of HCV transmission and hence they are not found as risk factors in the study. However, this does not mean that HCV is not transmitted sexually or vertically. The no-risk-factor group could also mean that there might be other modes of transmission for this virus.

6.4 EVALUATION OF DIAGNOSTIC ASSAYS
6.4.1 HBsAg ELISA Evaluation

Hepatitis B was the earliest recognised blood transmissible virus and has been screened for by hepatitis B surface antigen (HBsAg) testing since the early 1970s. However, current estimates suggest in a country like UK that the virus is still responsible for around 40 cases of post-transfusion hepatitis annually. These arise because HBsAg disappears from the bloodstream for a period in the early stages of infection, and during this time the test is
ineffective. One way of closing this window would be to adopt a national policy of screening for hepatitis B core antibody, as they do in the USA and much of Europe (Kay et al, 1995).

HBsAg detection depends not just on its presence or absence but also on its titre. Because, even when it is present in the serum, but at a low titre, certain kits are not able to pick them as positive due to their low sensitivity. Evaluation of the existing kits itself becomes very essential due to the gravity of the situation. The present study is based primarily keeping the above mentioned objectives in mind and finding the best kit in terms of sensitivity, specificity, positive and negative predictive value.

There have been many reports on these type of studies previously. McCready et al (1991) and Mutlu and Kumdali (1984) had conducted a comparative study to detect HBsAg by ELISA, RIA and PHA. They found that ELISA is the most sensitive commercially available test for HBsAg. Even in the present study, it was observed that the Lisadex and Uniform II had 100% sensitivity and specificity with a HBsAg detectability upto 0.3 and 0.4 ng/ml of serum. In one of the evaluation studies by Babes et al (1991), when 7 commercial HBsAg diagnostic kits were used, it was observed that there was no significant difference in sensitivity between the various kits. Even in the present study, though some of the kits picked up false-positives and false-negatives, there was no significant difference in the HBsAg positivity between the various kits. The difference in HBsAg positivity may not be significant between the various assays but what is important is whether a
particular kit is able to pick up all truely-infected cases as positive and non-infected cases as negative. This is where the sensitivity and specificity of a particular assay plays a major role in its successful implementation.

The incidence of false-negatives by ELISA in the present study was found to be 0.5% by Biotest, Bioelisa and Trans EIA. This figure is low when compared to previous reports (Kacaki et al, 1978; Vandervelde et al, 1978; Hyland et al, 1979), where the false-negativity was reported between 3.8%-10.6%. Similarly, the false-positivity in the present study is very low (1.5%) when compared to the previous findings of Kacaki et al (1978) who had reported a false-positivity between 2.2% -3.7%. The reduction in false-negativity and false-positivity in the present study might be due to the fact that in the 70's the kits were not that well developed and the assay systems that are available today are all in the third generation.

6.4.2.1 Comparative evaluation of HCV assays

Hepatitis C has to be considered as a fastly progressive infection than hepatitis B, since more than 50% of the HCV positive cases lead to chronic disease. Diagnosis and intervention at an early stage of this viral infection becomes very important. Since the discovery of HCV, there had been an array of first, second and now, the third generation of immunoassays for the detection of anti-HCV. Most of the early epidemiological and diagnostic studies were carried out using the so-called first generation ELISA, which detected antibodies to c100-3, representing a part of the NS4 region of the HCV genome. The presence of antibody to c100-3 proved a good marker for infection with
HCV in both post-transfusion and sporadic NANB hepatitis (Alter et al, 1989; Esteban et al, 1989; MJ Alter et al, 1990). It soon became evident that there were problems with the specificity of the assay, especially when screening low-risk groups such as blood donors, in whom there was a substantial risk of false-positivity (McFarlane et al, 1990). False-positive results were also common in patients with hypergammaglobulinaemia, and in stored tropical samples (McFarlane et al, 1990; Ellis et al, 1990). The assay also lacked sensitivity in that, anti-c100-3 was not present in the sera of all individuals with past or present HCV infection (Van der Poel et al, 1991; Marcellin et al, 1991) or during the early clinical phase of the illness and can take up to 1 year after elevation of transaminases to become detectable (Alter et al, 1989).

The deficiencies in the first-generation assays were responsible for the development of second generation tests, which incorporated two extra HCV-derived recombinant proteins, which increased the sensitivity and specificity of the assay (Aach et al, 1991; McHutchison et al, 1992). The second generation ELISA (ELISA-2) still had some risk of false positives and missing out HCV-infected cases. As a result, the third generation ELISA (ELISA-3) came into existence with detectability of HCV antibodies to C, NS3, NS4 and NS5 regions of the virus. This assay had much better specificity and sensitivity than the ELISA-2 (Goffin et al, 1994). In the meanwhile, the danger of lack of specificity of the ELISAs had necessitated the development of supplemental assays for the confirmation of positive results. The most widely used supplemental assay has been the Recombinant immunoblot assay (RIBA), in which recombinant proteins are coated on nitrocellulose strips, which enable
the detection of anti-HCV antibodies. The RIBA also had a similar developmental history as that of the ELISA, with 5-1-1 and c100-3 in the first generation (RIBA 1.0), 5-1-1, c100-3, c22 and c33c in the second generation (RIBA 2.0) and c100-3, c33c, c22 and NS5 in the third generation RIBA (RIBA 3.0). The RIBA 3.0 is by far the most sensitive and specific supplemental assay (Zaaijer et al, 1994; Lavanchy et al, 1994, Pawlotsky et al, 1994).

There have been a lot of evaluatory studies, comparing the different generations of ELISA and RIBA with the RT-PCR, conducted in other parts of the world but very few from the Indian subcontinent. Nanda et al (1994) in their study compared the ELISA-2 with the RT-PCR. The present study reports for the first time a comparative evaluation of the ELISA-2, ELISA-3 and RIBA 3.0 with the RT-PCR in India. It was seen that, of the initial 108 samples, only 4 were positive by all the four assays (ELISA-2, ELISA-3, RIBA 3.0 and the RT-PCR), an observation similar to that reported by Lavanchy et al (1994). The ELISA-3 had picked up additional 5 anti-HCV positives which were also positive by the RIBA 3.0 and RT-PCR. 2 samples which were positive only by the RIBA were also found to be positive for HCV-RNA. The anti-HCV positivity correlates well with the HCV-RNA positivity as seen in earlier reports. However 7 anti-HCV positive cases picked by the ELISA-3 and RIBA 3.0 were found to be negative for HCV-RNA. This observation has been reported by Zaaijer et al (1994) and has been attributed to viraemia below the PCR detection limit or past infection or a false positive reaction. One sample, which was negative by the ELISA-2 and ELISA-3, was found to be RIBA indeterminate and positive for HCV-RNA. Similar observations have been
recorded in earlier studies (Lavanchy et al., 1994; Vernelen et al., 1994) where comparison was conducted between Murex VK48, RIBA 3.0 and the RT-PCR and found that some of the samples found negative by Murex were either positive or indeterminate by RIBA.

The ELISA-2 did not pick up any false positive cases in the present study but missed out substantial number of cases. While comparing the performances of the three anti-HCV assays, it was seen that the positivity by ELISA-3 and RIBA 3.0 was comparable (p>0.05). Whereas, the anti-HCV positivity by ELISA-2 was significantly low when compared to ELISA-3 and RIBA 3.0 (p<0.001). Hence the ELISA-2 was not considered as a useful screening tool for anti-HCV.

In the second part of the study, where 213 samples were screened by the ELISA-3, RIBA 3.0 and the RT-PCR, it was seen that 87 cases were positive by both ELISA-3 and RIBA 3.0 of which 80 (91.9%) were found positive for HCV-RNA. Such high RNA positivity among anti-HCV cases has been previously reported by Lavanchy et al (1994) and Zaaijer et al (1994). The findings that ELISA-3 anti-HCV positive cases may be indeterminate by RIBA 3.0 or the ELISA-3 negative cases may be positive or indeterminate by the RIBA are well supported by similar observations by Lavanchy et al (1994). 13 cases, which were found negative for anti-HCV by ELISA-3 and RIBA 3.0, were found to be positive for HCV-RNA. HCV-RNA positivity in anti-HCV negative cases has been previously reported and attributed to the fact that either the antibodies are absent or in low titres which indicates that the sensitivity of
ELISA-3 and RIBA 3.0 is not yet optimum (Goffin et al, 1994). The presence of HCV-RNA in the anti-HCV cases in the second part of the study is due to the fact that the study group consisted of AVH, CRF and IVDU cases; and the absence of HCV-RNA positivity in the anti-HCV negative cases in the first part of the study is due to the fact that HCV-RNA positivity is low in chronic hepatitis as reported earlier (Casselman and Alt, 1996). The anti-HCV positivity and HCV-RNA positivity by both the assays was comparable (p>0.05), which means that ELISA-3 is as good as RIBA and could be used as a screening assay in labs where RIBA is not affordable.

6.4.2.2 RIBA Seroreactive pattern

With the introduction of RIBA the diagnosis of HCV has been made much easier and the RIBA seroreactive pattern itself has generated much more information than just the HCV positive or negative status of an individual. The RIBA 3.0 has four antigens-c100, c33, c22 and NS5 coated onto the nitrocellulose strips. To consider a specimen HCV positive, there should be reactivity to two or more of these 4 bands. There have been various patterns described by several workers earlier. In a study by Lavanchy et al(1994), comparing the RIBA 2.0 and RIBA 3.0 and the Western blot it was shown that majority of the samples had reacted to all the 4 antigens, followed by reactivity combination of c100, c33 and c22 and c33 and c22. Reactivities in other various combinations were seen in minor percentage of cases.
Even in the present study maximum reactivity was seen in the combination of c100, c33, c22 and NS5 and c100, c33 and c22; followed by the combination of c33 and c22. Other combination reactivities were to a lesser percentage. In the reactivity pattern, higher HCV-RNA positivity was seen in cases positive for all the 4 antibodies and in cases having positivity to c33 or c22 than c100 or NS5. Similar observations, highlighting the significance of c33 and c22, as indicative of the viraemic phase of HCV have been reported previously (Peignoux et al, 1992; Lavanchy et al, 1994; ; Pawlotsky et al, 1994; Pawlotsky et al, 1996).

What is significantly relevant in the RIBA seroreactive pattern is the association between the reactivity pattern and the probable HCV genotype. In Scotland, it was found that infection with genotype 2 and 3 strains of HCV tended to occur without anti-c100 antibody and patients with type 3 infection tend not to have anti-c33 antibody (Chan et al, 1991). The discovery that 90% of type 1 infection but only 22% of genotype 2 and 3 infections were associated with anti-c100 antibody predicted an overall seroprevalence for anti-c100 of 56% in sporadic disease and a higher figure for clotting factor and IVDU cases because of mixed infections (McOmish et al, 1993). Another study by McOmish et al (1994) goes on to show that donors infected with HCV type 1 showed broad serological reactivity with all four antigens on RIBA 2.0, while infection with divergent HCV genotypes elicited antibodies mainly reactive to c33 and c22. Reactivities with antibodies to 5-1-1 and c100 were infrequent and generally weak. The seroreactive pattern could be correlated much closer to the subtype level; because it has been reported that genotype 1b has significantly
higher reactivity to c33 and c22 than genotype 1a (Alonso et al., 1994). In the present study it was seen that c100, c33 and c22 had an overall reactivity of 82.3%, 93.75% and 99.2% respectively which indicates that the genotype probably could be type 1b. This finding correlates well with that of a study by Valliammai et al. (1995), in which they had reported genotype 1 as the predominant genotype in Southern India.

6.4.2.3 RIBA Indeterminates

The third generation RIBA 3.0 was introduced in Europe in mid-1993 and since then has been widely used in virology laboratories and blood banks. Several studies showed that RIBA 3.0 was able to resolve most of the RIBA 2.0 indeterminate pattern (reactivity to only one band), observed in patients routinely tested for anti-HCV antibodies in hospital virology laboratories, into positive or negative (Lunel et al., 1993; Pawlotsky et al., 1994). However some indeterminate patterns by RIBA 2.0 remained unresolved by RIBA 3.0. The RIBA indeterminate patterns were mainly found in patients with severe immunosuppression attributable to human immunodeficiency syndrome virus (HIV) infection or organ transplantation or immunosuppressive drugs.

In the present study, it was seen that 58 (17.5%) of the RIBA reactive cases were RIBA indeterminate. As reported in earlier studies (Pawlotsky et al., 1996) majority of the "indeterminates" were against c33 (24.1%) and c22 (58.6%). There were no indeterminate cases against NS5 antigen. This could be due to the fact that NS5 does not contribute to the increase in sensitivity of the assay (as seen by the low reactivity of NS5 among true RIBA positives),
but the increased sensitivity is due to improved NS3 reactivity (Vernelen et al., 1994). c33 and c22 could be very good indicators of viral replication as evinced by 57% and 67.6% of HCV-RNA positivity respectively among the two groups in the present study and also by the previous workers (Dussaix et al., 1994; Pawlotsky et al., 1994; Pawlotsky et al., 1996). What is noteworthy is, none of the c100 indeterminate cases were found to have HCV-RNA in them, as reported in earlier studies too (Lavanchy et al., 1994).

The reason for the indeterminate status was investigated, as to see if there was an immunosuppression status faced by the host during the infection. The basis of this investigation was the results from previous studies conducted by Lunel et al.(1993) and Pawlotsky et al.(1994). RIBA indeterminate cases were more prone to have an immunosuppressed status, due to organ transplantation or HIV infection, than the RIBA reactive cases. In the present study it was seen that 20 (34.4%) of the 58 indeterminate cases were positive for anti-HIV antibody as compared to 5 (8.3%) of the 60 HCV positive cases (p<0.01). The HCV-RNA positivity was significantly higher in the anti-HIV positive RIBA indeterminates than in the anti-HIV negative RIBA indeterminates indicating that the HIV-HCV coinfection may suppress the expression of HCV seroreactive antibodies though not HCV-RNA.

6.4.2.4 Surrogate markers

In earlier years, despite the measures, post-transfusion hepatitis (PTH), usually of the non-A, non-B type, develops in 7% to 12% of recipients of blood products (Alter et al., 1972; Dienstag et al., 1983). Use of surrogate markers,
such as antibodies to hepatitis B core antigen (anti-HBc) and elevated serum alanine aminotransferase activity, was recommended to screen for non-A, non-B hepatitis and thus to reduce further the incidence of PTH (Alter et al, 1981). This concept of testing for ALT levels as surrogate markers evolved from a retrospective analysis of two studies (Aach et al, 1981; Alter et al, 1981) which reported a significant association between elevated ALT levels in the donor and the development of hepatitis in the recipient. These same studies were reanalysed and paradoxically showed a significant association between the presence of hepatitis B core antibody in the donor and the development of NANB hepatitis in the recipient (Stevens et al, 1984). It was presumed that subjects exposed to HBV might also be more likely to be exposed to the NANB virus.

A similar analysis was made in the present study. The efficacy of surrogate marker testing was analysed with that of the specific viral marker (anti-HCV/HCV-RNA) testing. It was seen that, of the 317 HCV positive cases only 61 (19.24%) had anti-HBc IgM positivity and of the 915 HCV negative cases 214 (23.4%) were positive for anti-HBc IgM. Comparing HCV specific markers and anti-HBc IgM (p<0.001) it was evident that anti-HBc IgM was a poor marker for indicating HCV infection. Likewise, the ALT elevation was observed in only 112 (35.3%) cases of the 317 HCV positive cases. This again failed to have any significance as ALT elevations were also seen in HCV negative cases and most of the HCV positive cases had normal ALT levels. None of the 9 blood donors, who were anti-HCV positive, were positive for anti-HBc or had an elevated ALT level. Higher ALT levels had significant correlation with higher HCV-RNA positivity (p<0.01).
The present study clearly indicates the limitation of elevated ALT levels as markers of HCV infection. It is a marker of liver damage in general and may occur for a number of reasons. The study also shows that not all HCV positive cases will have raised ALT levels and not all individuals with raised ALT levels will be suffering from HCV infection. ALT elevations by themselves do not prove the occurrence of HCV infection. Furthermore, in prospective studies of transfusion recipients, anti-HCV screening of donors has already been shown to be more predictive of HCV infectivity than detection of elevated ALT levels (van der Poel, 1989; Barrera et al, 1991; Dasarathy et al, 1992). Similar considerations apply to the use of anti-HBc as a "surrogate marker" for NANBH. Anti-HBc as a marker for NANBH is particularly non-specific in areas that are endemic for HBV infection.

6.5 HCV AND AUTOANTIBODIES

There are conflicting reports regarding the occurrence of hepatitis C antibodies in patients with autoimmune liver disease. Clearly ELISA for anti-HCV is prone to false-positive results in patients with high concentrations of immunoglobulins in serum (McFarlane et al, 1990). These false-positive anti-HCV antibodies in patients with anti-smooth muscle antibody (ASMA) may actually disappear with immunosuppressive treatment as globulin levels decrease (Schvarcz et al, 1990). In Japan, 80% of patients with chronic NANB hepatitis have circulating antibodies to a pentadecapeptide (Gor), an epitope of normal hepatocytes; this phenomenon may represent an autoimmune response peculiar to type C hepatitis (Mishiro et al, 1990).
Upto 50% of patients with type II autoimmune hepatitis (anti-liver kidney microsomal (LKM) antibody positive) are anti-HCV positive, and anti-HCV and anti-LKM in association may also represent another example of molecular mimicry (Manns, 1991). High frequency of autoantibodies in patients with chronic HCV infection has been reported by Clifford et al (1995). In their study they had showed the occurrence of anti-nuclear antibodies (ANA) and ASMA to a tune of 41% to 76%. In the present study, AMA and ASMA were seen 62% of the HCV-positive cases. The autoantibody positivity in HCV positive group was significantly higher than that of HBV positive group and the NBNC group (p<0.01). Similar observations have been made previously by Manns et al (1989) and Lohse et al (1994), who had also demonstrated that the HCV positive group had positivity only to ANA and ASMA and not to AMA. The autoantibody positivity was significantly high (p<0.001) in the liver disease group (44.6%) than the blood donor group (2%) a finding similar to reports previously (Mantelli et al, 1996).

Very few studies have been reported on the association of autoantibodies with the stage of liver disease and a particular virus. In one of the study, McFarlane et al (1986) demonstrated anti-LSP (antibody against a liver specific membrane lipoprotein) circulating in the majority of patients with acute hepatitis B and of more interest, the antibodies were found in majority of patients with CAH-type B but not in patients with CPH type-B. In the present study, it was seen that significantly higher autoantibodies were seen in CPH and CAH group of HCV positive cases than the Cirrhosis group (p<0.001), which could indicate that HCV may trigger autoantibody production earlier in the course of liver disease. The NBNC group had a low positivity initially but in the Cirrhosis stage the autoantibody positivity was high and
comparable with that of the HCV positive group. This could mean that in the absence of any viruses, the autoantibody production may take longer in liver diseases.

In a study by Manns (1991), it was seen that anti-HCV patients with autoantibodies were usually male and of older age than patients without anti-HCV. Similar observations have not been recorded in the present study regarding the gender but there has been a definite association between older age and increasing autoantibody positivity.

6.6 FOLLOW-UP STUDIES

The clinical course of liver disease is affected by the presence of viruses like HBV and HCV. In most adult cases of acute hepatitis B, serum HBsAg disappears within 3-4 months after exposure, but in about 5% of patients antigenaemia will be detected for more than 6 months. In contrast, 90% of babies who have been infected perinatally or within the first 5 years of life become HBV carriers and have little chance of spontaneous recovery during their lifetime. Whereas hepatitis C is known for its chronicity as more than 50% of the infected cases go in for chronic liver diseases (Seeff and Koff, 1986).

The present study has shown that 8 of the 9 AVH cases of type-B had recovered and one of them had slipped onto the carrier state, as he had the virus for more than an year. This finding is very similar to the existing literature that 5-10% of the HBV infected cases may become carriers (Seeff and Koff, 1986; Hoofnagle et al, 1987). One of the CPH cases turned HBsAg negative after 24 months. There was no HBsAg seronegativity in the CAH,
Cirrhosis and HCC cases even after 24 months which supports the earlier studies which suggest that HBsAg may persist lifelong in chronic liver diseases (Hoofnagle et al, 1987; Coppola et al, 1996a). In the present study the HBeAg positivity and the HBV-DNA positivity was high in the initial stage but decreased substantially after 24 months. This finding is supported by previous studies (Karayiannis et al, 1985; Wu et al, 1986) where it was shown that there is marked decrease in the HBV-DNA in the later stages of the chronic liver disease.

In the HCV follow-up, it was seen that 21 of the 25 HCV-positive cases had persistence of HCV-RNA for more than one year. This was a major difference observed between HBV and HCV cases in the present study. While in HBV positive cases, it was observed that 8 of the AVH cases and one CPH case turned HBsAg negative or had a seroconversion within a period of 6 months to 2 years. Whereas in HCV positive cases, either the HCV-RNA or the anti-HCV status persisted in all the cases. According to Tanaka et al (1993) and Coppola et al (1996b) the only marker that suggested recovery or responders, was the c100. In the present study it was seen that the c100 disappeared on follow up in two of the patients being treated with interferon. The HCV-RNA also became negative but with persisting anti-HCV reactive status. This observation is very much similar to previous findings by (Tanaka et al, 1993). Of the 7 RIBA indeterminates, 4 turned out to be true anti-HCV positives. Such phenomenon observed earlier by Pawlotsky et al (1994) has been reported to be due to temporary immunosuppression by organ transplantation, immunosuppressive therapy or HIV infection. The 2 AVH cases which progressed to CAH could have been as a result of higher chronicity rates of HCV than HBV (Alter et al, 1989).