Chapter 6

Discussion
6. DISCUSSION

6.1 HEPATITIS B VIRUS (HBV) AND HEPATITIS C VIRUS (HCV) COINFECTION IN HIV INFECTED PATIENTS

India is the most densely populated country in Asia and the second most populous country in the world. Since its independence from Britain in 1947, India has become self-sufficient in agricultural production, experienced rapid industrial growth and made great strides improving health care for citizens. However, India still faces enormous public health problems including a high prevalence of diarrheal disease, tuberculosis, malaria, hepatitis, and sexually transmitted diseases. The first reported cases of HIV infection diagnosed in India were among Madras commercial sex workers (CSWs) in May 1986 (Simoes et al., 1987) and the first AIDS case in India was reported to the ICMR in May 1986 in a transfusion recipient who had coronary artery bypass surgery in the United states (NACO, 1993). Of the global total of people who are living with HIV, 95% live in developing countries. India, where 3.97 million Indians are living with HIV/AIDS, has the highest number of HIV-infected persons in Asia.

The liver is an important organ which gets inflicted in patients with AIDS because of its frequent involvement in systemic infections and malignancies, and its tropism for the hepatitis B and non-A, non-B viruses (Lebovics et al., 1985). The prevalence of viral hepatitis B (HBV) or hepatitis C (HCV) in patients infected with human immunodeficiency virus (HIV) is common (McNair et al., 1992). In the present study on 500 HIV infected patients 9% were found to be positive for HBV (95% CI 6.5-11.5) and 2.2% of the cases were found to be positive for HCV (95% CI 3-3.5). Our HIV/HBV coinfection (9%) finding is similar to that of the reported prevalence of hepatitis B surface antigen (HBsAg) chronic carriage among HIV
infected individuals is 1.9 to 9% (Saillour et al., 1996). Among IVDUs, 90% of HIV infected individuals have evidence of exposure to hepatitis B (hepatitis B core antibody (antiHBc) positivity) and 60% also have evidence of past infection with presence of hepatitis B surface antibody (antiHBs) (Rodriguez-Mendez et al., 2000). Additionally, anti-HBc is more frequently found in HIV infected homosexual men compared to those who are HIV seronegative (72% vs. 31% respectively). Our analysis of the modes of acquiring HBV and HCV in the group of HIV/HBV and HIV/HCV revealed that heterosexual in 78% of cases while blood transmission and others was seen in 4% and 18% respectively in HBV coinfected groups. Where as in case of HIV/HCV coinfected, cases the risk factor analysis revealed that the heterosexual in 37% of cases while blood transmission, intravenous and others were seen in 27%, 27% and 9% respectively.

HIV and HBV share routes of transmission and thus up to 10% of HIV-infected individuals have chronic hepatitis B (Rustgi et al., 1984). According to different routes of transmission 4 - 94 % of the HIV infected patients present with detectable antibodies against hepatitis C. (Wright et al., 1994 and Dorruci et al., 1992) and 64-84% are positive for Hbc antibodies (Halder et al., 1991). In our HIV/HBV coinfected group 78% patients had the previous history of heterosexual promiscuity where as in the HIV/HCV coinfected group revealed that 37% had the heterosexual promiscuity, followed by 27% blood transmission, 27% intravenous and 9% of other risk. The prevalence of HCV infection among all HIV-infected individuals can be as high as 40% but this prevalence varies substantially among different risk groups (Dieterich et al., 1999). In most series the prevalence of HCV among HIV - infected intravenous drug users (IVDU) is 50-90% (Huemer et al., 1990).
Approximately 400 million persons worldwide have chronic hepatitis B, and between 500,000 and 1 million persons die annually of HBV related disease (Mahoney et al., 1999). In the United States, there are approximately 3.35,000 incident cases annually (Coleman et al., 1998) and about 1 million persons chronically infected. Another study (Rustgi et al., 1984) reported that up to 10% of all HIV-infected persons have HIV/HBV coinfection in the United States. Coinfection with hepatitis B virus (HBV) and HIV is common, with 70 to 90% of HIV infected individuals having evidence of past or active infection with HBV (Rodriguez-Mendez et al., 2000). There are about 150 million chronic hepatitis C (HCV) carriers throughout the world with an estimated global prevalence of 3% (range 0.1 - 5%) (EASL, 1999). In the United States nearly 2% of the population is infected with hepatitis C virus (HCV) (NIH Report, 1997). The prevalence of HCV in HIV-infected homosexual males is similar to that observed in HIV-negative homosexual males at 4-8% (Bodsworth et al., 1996). Although the rate of sexual transmission of HCV is low (<5%), this rate may be increased in the setting of coinfection with HIV (Thomas et al., 1996). In our HIV/HBV coinfected patients 87% were male and 13% were female populations. In the HIV/HCV coinfection 82% patients were male and 18% were female populations. Although in our study we observed predominance of males than the females in both groups, it was not statistically significant.

Despite detail reports were documented worldwide in association with HBV, HCV and HIV coinfection, only a few reports have been published regarding the coinfection in India. Sud et al. (2001) have reported 33.8% prevalence of HBV coinfection in HIV positive patients. Kumarsamy et al. (2002) reported a coinfection rate of 6% HBV and 4.8% HCV in HIV infected cases. Fourteen percent of HBV infected and twenty five percent of HCV infected patients had received blood
transfusion; fifty percent of HCV infected patients were injection drug users (IVDU). Kumar et al. (2003) reported that 2.9% co-infection of HBV and HIV in patients of liver diseases. Tankhiwale et al. (2003) reported 5.6% seroprevalence of HCV and 25.8% HBV in HIV infected patients, the coinfection rates were much higher than that of our findings. Although the HIV shares common route of infection with HBV and HCV, the HBV is known to be transmitted sexually where as the sexual transmission of HCV appears to be less efficient means, certainly less efficient than is the case for HIV (Wyld et al., 1997).

At the present rate of transmission of HIV, India will have the largest number of HIV-infected individuals of any country in the world by the end of this decade. Since, there is similarity in the modes of infection between HBV, HCV and HIV infection there is every possibility that HIV infected person may acquire HBV and/or HCV. It is thus clear that apart from other infections, HIV infected individuals have a high probability of getting coinfected with HBV and/or HCV. Because only limited data is available, comprehensive and well designed epidemiologic surveys are urgently needed to adequately characterize the HIV/hepatotropic virus epidemic in India to help implement targeted and effective educational and prevention oriented programs.

6.2 STUDIES TO ANALYSE THE INTERACTION BETWEEN HUMAN IMMUNODEFICIENCY VIRUS (HIV) AND HEPATOTROPIC VIRUSES

Coinfection refers to infection with two or more different disease causing organisms. There is increasing evidence from the molecular to the clinical level that
the effects of HIV infection can be modified by coinfection with other viruses. The hepatitis viruses, A through D are prevalent among patients at risk for HIV infection. Several properties of the hepatitis A through D viruses and the HIV suggest that coinfections are frequent and clinically important. Like HIV, HBV, HCV, and HDV all can be spread parenterally and sexually (Solomon et al., 1988). Hepatitis A virus (HAV) infection is prevalent among homosexual men (Corey et al., 1980) and intravenous drug users, which are the groups at high risk for HIV infection in this world. In addition, HBV, HCV, and HDV can cause chronic infection. Thus, coinfection may be expected to occur relatively frequent. From the literature accumulated over the two decades of the AIDS epidemic, it appears that HIV infection alters the course of hepatitis B, C, and D. Interactions among the hepatotropic viruses and HIV are complex and suggest that mechanisms associated with the immunologic responses to these viruses have not been fully elucidated. It is possible that as patients with AIDS live longer, issues regarding hepatotropic virus infections may become more important.

6.2.1 Correlation between HBV and HCV viral markers profile and HIV disease groups (CDC-1993 revised)

Coinfection with HIV and hepatotropic viruses cause complex interaction. HIV induced impairment of the cell mediated immunity cause higher replication of hepatotropic viruses (Housset et al., 1992). Additionally HBV leads to enhanced transcription of HIV through an NF-κB element in the long terminal repeat of HIV (Twu et al., 1989). HCV also activates through cytokines like tumor necrosis factor-α HIV replication. However, the impact of these coinfections on the clinical outcome of HIV infected patients is unclear. Earlier reports suggest that coinfection with either HCV (Martin et al., 1989 and Soriano et al., 1995) or HBV (Eskild et al., 1992) accelerate the clinical course of HIV infected patients. In
contrast, other studies have not revealed significant progression of the disease in HIV infected patient with either hepatitis B or hepatitis C (Wright et al., 1994) coinfection. Thus it is unclear whether improvement of the long term outcome of HIV positive patient is influenced by coinfection with a hepatotropic virus.

The apparent relation between HBV infection and AIDS is of particular interest. Hepatitis B virus markers were more prevalent in patients with AIDS compared with patients without AIDS. Anti-HBs which typically reflects immunity, did not differ between patients with or without AIDS, where as HBsAg which indicates chronic infection, was more than twice in the AIDS group. This association has at least two potential explanations. First, persistant HBV infection may accelerate the progression of HIV related disease. The similar survival of HBsAg positive and negative patients, however militates against this possibility. Rather, the findings suggest that patients with the more advanced immunosuppression characteristic of AIDS may be less likely to clear HBV infection after exposure or more likely to reactivate latent HBV infection, or both (Scharschmidt et al., 1992).

Our study showed that in HIV seropositive patients, coinfection with hepatitis B or hepatitis C is frequent. In coinfected patients, the HBV-DNA and HCV-RNA positivity was higher. The effects of HIV on the course of chronic HBV infection have largely been assessed retrospectively. Comparisons have been made of USG/CT scan findings of liver, biochemical, and serological parameters of HBV/HCV activity in patients with concurrent HIV infection vs. those without such infection. In the HIV infected patients many factors other than coinfection with hepatotropic viruses may influence the outcome, such as age, access to medical care, antiretroviral medication (Chaisson et al., 1995), and nutritional status (Suttmann et al., 1995). Sex, race, IVDU or socioeconomic status (Chaisson et al., 1995) have no influence on the course of HIV infection. HIV seropositivity was associated with
a significantly higher prevalence of serum HBeAg and HBV DNA. In the HIV disease group of HIV/HBV coinfected cases, the HBsAg positivity was higher in both group B (48%) and group C (42%) compared to group A (10%) of HIV disease group. A higher degree of immunodeficiency, as shown by the CDC 1993 revised classification of HIV disease group (group A, B and C), is associated with a higher rate of HBeAg in HBsAg positive patients; in addition, the HBV replication increases with the HIV disease progression. HBe positivity was 25%, 47%, and 71% were in HIV disease group A, B and C respectively, whereas anti-HBe positivity was 75%, 53% and 29% were under HIV disease group A, B and C respectively. The HBeAg and anti-HBe positivity pattern of our HIV study group very clearly shows that, both the HBV serological markers (HBe and antiHBe) were inversely proportional when HIV disease progresses in HIV/HBV coinfected patients. Statistically there is a significant trend between HBsAg positivity and HBV-DNA positivity between the three CDC defined HIV disease groups at 95% level of significance (p=0.03).

In our study in the HIV/HCV coinfected groups, the HCV-RNA positivity was found to be higher in Group-C (71%) than group B (66%) but only one patient in group A showed positive for HCV-RNA also. These observations confirm previous reports of increased viral replication of hepatotropic virus in immunocompromised patients (Housset et al., 1992). The U.S. Public Health Service (USPHS) and the Infectious Diseases Society of America (IDSA) recommended screening with EIAs for all HIV-infected individuals to detect anti-HCV (USPHS/IDSA-1999). However, a single negative antibody assay may not sufficiently exclude HCV in HIV-infected individuals. The increased rate of false negative antibody testing in the presence of HIV has been attributed to several factors, including a possible lack of HCV antibody production with
immunosupression, more rapid decline in HCV antibody titer, possible interaction between the two viruses, and an anti-HCV seroconversion into a negative state. In our study out of 300 anti-HCV negative HIV infected patients, two patients showed HCV-RNA positivity and their CD4 counts were <400 cells. Therefore, while patients should be screened by EIA assay, measurement of serum HCV-RNA by highly sensitive reverse transcriptase PCR may be required for detection of HCV in individuals with undetectable antibody and other evidence of chronic liver disease (USPHS/IDSA-1999).

The liver is an important organ to evaluate in patients with AIDS because of its frequent involvement in systemic infections and malignancies, and its tropism for the hepatitis B and non-A, non-B virus, agents that bear epidemiologic similarities to AIDS. Lebovics et al. (1985) reviewed the clinical data and histological findings of patients with AIDS. The most common histological finding was macrovesicular steatosis. McDonald et al., (1987) confirmed the hypothesis that chronic HBV carriers positive for anti-HIV have serological and immunohistochemical findings similar to chronic HBV carriers on immunosuppressive drugs. The increased viral replication and altered HBV envelope (pre S2) expression in AIDS patients indicate that HIV significantly affects the HBV life cycle and the host ability to clear HBV infection. If this holds true, more HBV infection and more chronic carriers would be expected has the AIDS epidemic expands. Such a profile would have worrisome public health implications since more chronic liver diseases, including HCC, would be expected as the mortality rate associated with HIV is reduced.

Gilson et al., (1997) strengthen the evidence for a significant of HIV infection on the natural history of chronic HBV infection, which by prolonging the period of infectivity could have an important impact on the epidemiology of HBV
infection. These interaction may be important for the case of patients in Europe or North America, but they have even greater significance for the area of world where HBV prevalence in high, which are also the region with the major burden of HIV disease-Africa and Asia. Hence, the increased infectivity could have a major effect on the spread of HBV infection both horizontally and vertically. They have not seen any of the consistent effect of HBV infection on the rate of progression of HIV disease.

Recent in-depth studies of the immunological aspects of HCV infection have revealed a CD4 lymphocyte response against viral proteins in chronically infected patients, despite the inability to clear the virus; however, the virus does not seem to affect the number of peripheral blood CD4 cells. In one longitudinal study of HIV seropositive persons belonging to different transmission categories, found that HIV-HCV coinfection did not influence the progression of HIV disease to AIDS and did not induce a more severe immunosuppression (Dorrucci et al., 1995). Finally author concluded that HIV-HCV coinfection does not seem to accelerate the course of HIV disease and the dual infection does not necessarily imply a faster development of HIV induced immunosuppression.

In one study by Ghany et al. (1996) observed that there was a difference in HCV-RNA levels between anti-HIV positive and anti-HIV negative patients, although HCV-RNA levels were significantly higher in anti HIV positive patients with CD4 counts <200 mm$^3$ ($p=0.008$), there was an inverse correlation between HCV-RNA levels and CD4 counts but no correlation was found between HCV-RNA and serum aminotransferase levels. HCV replication appears to be increased in patients with severe immunodeficiency secondary to progressive HIV infection. However, there was no correlation between HCV-RNA and serum ALT level suggesting that HCV is not directly cytopathic.
In another study by McDonald et al., (1987) observed that percentage reduction of HBV-DNA was significantly less in the anti-HTLV-III positive group in comparison to the anti-HTLV-III negative group. They also found that homosexual men with HBeAg positive CLD who are anti-HTLV –III positive appears to be less responsive to the direct antiviral and immunomodulatory effects of recombinant interferon-α2a. HBe/antiHBe seroconversion, with loss of HBV-DNA, is associated with clinical and biochemical improvements (Hoofnagle et al., 1981) and with a decrease in the inflammatory activity in the liver. Bodsworth et al., (1989) suggested that an increased infectivity for chronic HBV infections in HIV positive persons regardless of their clinical state or laboratory evidence of immune suppression, that may have implications for hepatitis policies, and epidemiologic studies should be considered to monitor a possible increase in the spread of HBV among population at risk for HIV and HBV. The demonstratable increase in viral replication in HIV positive individuals may also have therapeutic implications for chronic hepatitis B.

HIV infection facilitates HCV replication and leads to more severe liver damage. Serum HCV-RNA levels have been shown to correlate with some features of hepatic inflammation and more rapid clinical progression of liver disease seen in HIV seropositive hemophilic patients (Eyster et al., 1993). They speculate that liver pathology in those patients is due to viral rather than immune mediated damage, but clearly disease progressions are likely to be complex. In another study Wright et al., (1994) have demonstrated that HCV infection is several times more common in non-intravenous drug using HIV positive patients than in volunteer blood donor, suggesting that sexual transmission of the virus occurs. This finding further suggest that like HBV infection, HCV infection does not influence survival in these HIV positive patients.
In hemophilic patients, it has been shown that the prevalence of HCV infection in those who received pooled plasma products before HCV screening was close to 100% (Watson et al., 1992). Early concentrate were not subjected to a viral inactivation procedure and many recipients have been infected with both HCV and HIV (Watson et al., 1992). Coinfection with HCV and HIV also occurred in patients who received blood transfusion before the reduction of an antibody to HIV and HCV. Since all the blood donors in India are now screened for both anti-HIV and anti-HCV, this route of transmission has been virtually eliminated.

Housset et al. (1992) suggested that chronic HBV infection can be associated with severe liver damage in HIV positive drug abusers and homosexuals. HIV infection does not seem to attenuate and may even worsen–HBV chronic liver damage. The viral replication and host’s immune response are probably important factors that influence the outcome of hepatitis B infection. However, it is difficult to evaluate their respective roles because of continual interplay that can vary during the course of HBV infection. The long-term effect of immunodeficiency on the outcome of hepatitis B infection remains to be evaluated. Cell mediated immunity is considered one of the most important factors modulating viral replication in chronic HBV infection and immunosuppressive therapy is known to enhance viral replication (Hoofnagle et al., 1982). Housset et al. (1992) observed that the overall rate of HBV-DNA positivity did not differ between HIV positive and HIV negative homosexual subjects. The effect of HIV status on the severity of HBV related chronic liver disease in homosexual is indeed controversial; some authors (Krogsgaard et al., 1987 and McDonald et al., 1987) have reported significantly lower aminotransferase activity in HIV positive subjects but others have not (Perrillo et al., 1986; Rector et al., 1988; Bodsworth et al., 1989; Krogsgaard et al., 1987 and McDonald et al., 1987).
In our study, the overall mean of HIV-RNA load in HIV/HBV coinfected patients was higher (276312.9 ± 478580 copies/ml, Mean ± SD) than that of HIV/HCV coinfected patients. The HIV viral load in HIV/HCV (91143.24 ± 100147.6 copies/ml, Mean ± SD) patients was lower than HIV/HBV and HIV alone-infected patients (135247 ± 386789 copies/ml, Mean ± SD). The HIV-RNA viral load difference was however not significant in our study groups. In the overall analysis of the CD4, and CD8 counts in the HIV/HCV coinfected patients had lower than HIV and HIV/HBV coinfected patients, but the difference was not statistically significant. This could probably due to small number of cases, wide range of CD4, CD8 counts and the HIV cases being in different stages of HIV disease.

In the previous study by Kumarasamy et al. (2001) on human immunodeficiency virus disease progression in south India, difference in HIV-RNA viral load (copies/ml) and CD4 cell count was not seen between HBV/HIV and HIV/HCV coinfected patients. In our study we analysed the comparison of HIV viral load in HIV/HBV and HIV/HCV coinfected patients with respect to their viremic (HBV-DNA and HCV-RNA) status. The significant difference of HIV-RNA viral load was not seen in the HBV-DNA positive and HBV-DNA negative patients and the difference was not seen between HCV-RNA positive and negative patients also.

The escalating HIV epidemic in Asia the region with the highest background prevalence of chronic HBV infection will substantially expand the pool of people with HIV-1 HBV coinfection over the next decade.
Biochemical liver function parameters in HIV patients and the impact of hepatitis B virus (HBV) and hepatitis C virus (HCV) coinfection

Patients with acquired immunodeficiency syndrome (AIDS) commonly have clinical and histological hepatic abnormalities (Lebovics et al., 1985 and Reichert et al., 1983). Human immuno deficiency virus (HIV) infection itself does not appear to have direct effects on the liver (Lebovics et al., 1985).

In our analysis of the LFT markers (transaminases) profile in the patients who were infected with HIV and in HIV/HBV coinfected patients had revealed that the coinfected patients and patients infected with HIV alone had similar ALT and AST (ALT p=0.662 & AST p=0.290). However the analysis of the LFT (transaminases) markers profile in the patients who were infected with HIV and HIV/HCV coinfected patients had revealed that the patients with HIV/HCV coinfection had significantly higher ALT and AST than the patients with HIV alone (ALT p=0.0.05 & AST p=0.03). Rector et al. (1988) observed lack of relationship between serum AST concentration and indices of cellular immune function and HBV replication. They suggested that other factors determine the severity of hepatic inflammation in chronic HBV infection. Perillo et al., (1986) have reported that HIV positive homosexual men have less hepatic inflammation and more rapid hepatitis B viral replication than either HIV negative homosexual men or heterosexual subjects with chronic hepatitis B infection. Krogsgaard et al. (1987) found that serum AST levels were lower and serum HBV-DNA levels higher in male homosexual patients with chronic HBV infection and antibody to HIV, compared to similar without antibody to HIV. The common features observed in anti HIV positive subjects with chronic HBV infection are less severe liver injury, lower ALT levels, HBeAg positivity and serum HBV-DNA values higher than in anti HIV negative controls.
This suggests that these patients are less immunologically responsive to HBV antigens even in the absence of clinically evident immunodeficiency (Perillo et al., 1986 and Krogsgaard et al., 1987). Mai et al. (1996) concluded that there was no over influence by HIV or the treatment there of on the course of chronic HBV infection in a population of homosexual man. In HIV infected patients, death from AIDS predominated; hence, the main target for the therapy should be HIV rather than HBV. Sulkowski et al. (2000) observed that HIV infection is associated with higher HCV-RNA level and a more rapid progression of HCV related liver disease, which leads to an increased risk of cirrhosis. HCV infection may also impact the course and management of HIV disease, particularly by increasing the risk of ART-induced drug hepatotoxicity. Thus, chronic HCV infection acts as an opportunistic disease in HIV-infected persons, because the incidence of infection is increased and the natural history of HCV infection is accelerated in coinfected persons.

Benhamou et al. (2001) concluded that chronic use of ART containing PI together with reduction of alcohol consumption and maintenance of high CD4 count could have a beneficial impact on liver fibrosis progression in HIV/HCV coinfected patients. Graham et al. (2001) observed that the risk of severe liver disease is significantly increased in patients who are coinfected with HIV and HCV, compared to those patients who have HCV monoinfection.

In one aspect of our study, the comparison of the evolution of transaminases (ALT, AST) in HIV/HBV and HIV/HCV coinfected patients with respect to their viremic (HBV-DNA and HCV-RNA) status were analysed for the period of 24 months at an every 6 months interval follow up. In the HBV-DNA positive and negative cases of HIV/HBV and the RNA positive and negative of HIV/HCV coinfected patients analysed the difference in the ALT and AST was not seen in at any stage of the follow up period. But, in the inter group analysis of both
HBV-DNA positive & negative group of HIV/HBV coinfected cases were compared with HIV alone had revealed that the coinfected group had slightly higher than that of only HIV infected group but statistically the difference was not significant (p=>0.07). In the HIV/HCV coinfected group compared with HIV alone the ALT and AST were significantly higher in both RNA positive and RNA negative cases. (p=<0.05). Between the RNA positive and DNA positive group analysis revealed that the RNA positive HIV/HCV coinfected patients had slightly elevated ALT and AST compared to the HBV-DNA positive patients but the difference was not significant (p=>0.216). It seems that among the coinfected group, the HCV coinfected patients are highly prone for developing the hepatotoxic effect than HBV coinfected group. Boldorini et al. (1997) concluded that hepatitis C, coinfection with HIV showed tendency towards a lower response to IFN, although this did not reach statistical significance; however more of the HIV positive patients developed cirrhosis during the follow up and this should be considered in clinical management of such patients.

We analysed ALT, AST in all the three groups of patients based on their anti retroviral therapy status. It revealed that in the 24 months follow-up, the ALT and AST variation was not seen between ART group and naïve group of the HIV/HBV coinfected patients but in the later stage the statistically significant difference between these two groups was seen only by the 24th month (ALT p=0.05, AST p=0.01). In the HIV/HCV coinfected patients the ALT and AST enzyme elevation was seen compared to the normal values irrespective of ART status. The ALT and AST difference was seen in the groups of HIV/HCV ART compared to HIV ART (statistically significant difference was seen in the 6th month only) and ART group of HIV/HBV. But the statistical significance was seen only in the base as well as by the 6th month (p<0.04). This observation very clearly explain that care
should be taken on HCV coinfectected patients since they seem to have persistent liver damage irrespective of their ART status.

Colin et al. (1999) observed that anti-HIV positive patients and anti-HIV negative patients did not behave differently in serum aspartate transaminase activity, bilirubin, prothrombin, and histological activity index. The anti-HIV patients had lower ALT (p=0.0001), lower albumin (p=0.009), and higher serum HBV DNA levels (p=0.01), they also found that the higher prevalence of cirrhosis in anti-HIV positive patients (p=0.04). They have concluded that homosexual men with chronic hepatitis B, HIV infection is associated with higher level of HBV replication and a higher risk for cirrhosis without increased liver necrotic-inflammatory process.

Cribier et al. (1995) observed that mean ALT value of coinfectected patients was higher than the HIV negative group, but the difference was not statistically significant (p=0.08). They were not able to find a correlation between the ALT value and the HCV viremia. On the other hand, it cannot be excluded that the severity of liver disease described by others (Martin et al., 1989; Eyster et al., 1994 and Berk et al., 1990) in case of coinfection with HIV and HCV could be due to factors other than HCV viremia. Finally, they have concluded that a striking increase in HCV viremia in patients coinfectected with HIV. The HCV RNA load was neither correlated with HIV load nor with CD4 count, suggesting that indirect factors present in HIV infected patients could play a role in the enhancement of HCV replications.

Flares of liver enzymes in HIV-HBV coinfectected patients receiving highly active antiretroviral therapy (HAART) can arise from one or more of the following causes, so a careful evaluation is required to analyse the etiology prior to discontinuing or changing an effective HAART regimen. First, HBV infection clearly increases the risk for toxicity from antiretroviral medication (Sulkowski
et al., 2000); however, only a few experience a severe, reversible hepatotoxicity (ALT > 5 X the upper limit of normal (ULN) usually with in the first 6 months of starting a regimen. Coinfected patients are also at increased risk for the rare condition of hepatic steatosis and lactic acidosis from the nucleoside analogues, which can occur after years of therapy. Second, a flare may herald HBeAg seroconversion, so it is not unreasonable to continue HAART if this is a possibility. Third, immune reconstitution has been reported to lead to ALT elevations in patients with chronic hepatitis B (Velasco et al., 1999). Fourth, there have also been several case reports of reactivation or exacerbations of HBV after discontinuing lamivudine as part of a HAART regimen (Bessesen et al., 1999), and it is well described in the development of HBV resistance to lamivudine (Bessesen et al., 1999). Fifth, reactivation of HBV replication has also been described in the setting of HAART independent of lamivudine withdrawal or resistance (Manegold et al., 2001). Last, superinfection with another hepatotropic virus must be considered.

In HIV/HBV coinfected cases HIV related immunosuppression may reduce liver necro inflammatory lesions and serum alanine transaminase (ALT) level in HBV infected patients (Perillo et al., 1986). HBV infection has been shown to be associated with more severe liver fibrosis in HIV coinfected patients (Colin et al., 1999) which may increase mortality. Interferon alfa therapy, which was previously used as the most effective therapy in chronic HBV infected patients, has been reported to be poorly efficient in HIV infected patients, with the HBe seroconversion rate close to 0% (Wong et al., 1995). However, few cases of persistent interferon induced HBe seroconversion have been reported in HIV infected patients (Hoofnagle et al., 1998 and Martino et al., 1996) and the factors associated with the response to interferon therapy have never been well documented in HIV-HBV coinfected patients.
Brinker et al. (2000) observed that HIV-1 infected patients coinfected with HBV or HCV were at considerably higher risk of developing liver enzyme elevation (LEE) when HAART was initiated compared with patients without coinfection, but it is usually not necessary to modify antiretroviral therapy. The serum aminotransferase activity is lower in HIV seropositive patients with HBV infection (Bodsworth et al., 1989 and McDonald et al., 1988). This response does not always correlate with the stage of HIV disease. Sud et al. (2001) found that there was no difference in the transaminase level among the HBV positive and HBV negative patients in the HIV infected group. Finally the authors suggest that the absence of liver biopsy would be difficult to comment on the presence of chronic liver disease in the patients. The pathogenesis of hepatocellular damage by HCV is poorly understood. As with chronic hepatitis B infection, evidence is emerging that liver damage may be mediated by the immune reaction to infected hepatocytes, rather than by the virus itself (Koziel et al., 1992). However, the demonstration of HCV antigens in liver biopsies and the correlation between levels of viremia and degree of lobular inflammation suggest that HCV may be directly cytopathic to liver cells (Yuki et al., 1993). Eyster et al. (1994) confirmed the findings favor the mechanism of direct cytopathicity rather than cellular immune reactivity as the cause of hepatocellular damage in person with chronic HCV infection. Eyster et al. (1994) summarized that HCV-RNA levels are significantly higher in HIV positive than HIV negative multitransfused hemophiliacs. HCV load increase over time, is enhanced by HIV, and further increases as immune deficiency progresses. HCV-RNA levels are strongly associated with AST levels.

HIV/HCV coinfection can complicate HIV treatment due to adverse drug effects. These complications are of two types: 1. Liver specific side effects of antiretroviral drugs (hepatotoxicity), which may be worse in people with existing
liver damage due to chronic hepatitis. Many anti-HIV drugs are metabolized by the liver. When drugs are taken in high doses and especially when different drugs are combined, they can cause liver injury. This is especially likely in patients who have existing liver damage due to chronic viral hepatitis or other factors such as heavy alcohol consumption. Drug related liver injury is often signaled by increased levels of liver enzymes, in particular ALT and AST. 2. Drug interaction—in which anti HIV and HCV drugs that have similar side effects produce intensified (additive or synergistic) adverse events when the drugs are used together. Mitochondrial toxicity refers to drug-induced damage to small organelles within cells that are involved in energy production. Interestingly, high blood fat levels—one of the most worrisome side effects associated with anti-HIV drugs—appear less likely to occur in HIV/HCV coinfected individuals.

As liver failure becomes a more common cause of illness and death in people with HIV/AIDS, it is increasingly important to manage hepatotoxicity related to antiretroviral drugs and this is especially true for people with existing liver damage due to chronic hepatitis. Since the management of HIV, HBV and/or HCV coinfection can be complex, the care of coinfected people ideally should be managed by physicians who have experience with both diseases or by teams that include both a hepatologist (liver disease specialist) and an infectious disease expert.

6.2.3 The opportunistic infections in HIV patients and the impact of hepatitis B virus (HBV) and hepatitis C virus (HCV) coinfection

In our study we assessed the impact of hepatotropic viruses coinfection on HIV associated opportunistic infections (OPI) in HIV, HBV and/or HCV coinfected patients. The HIV disease is a continuum of progressive damage to the immune system from the time of infection to the manifestation of severe
immunologic damage by opportunistic infections (OI), neoplasms, wasting, or low CD4 lymphocyte count that define acquired immunodeficiency syndrome (AIDS). Opportunistic infections can occur in all organs by a variety of microbes. The spectrum of opportunistic infections varies from country to country. The commonest opportunistic infection in Italy is *Pneumocystis carinii* pneumonia whilst cytomegaloviral infection is the most common in Mexico (Mohar et al., 1992) and tuberculosis in Africa and in India (Kumarasamy et al., 1995). Over the past several years, effective antiretroviral medications have increased the life expectancy of HIV-infected persons, resulting in a changing spectrum of HIV–associated diseases. Chronic liver disease from hepatitis C virus (HCV) has been recognized as a HIV associated opportunistic infection (CDC 1999), and hepatitis B is being included. Opportunistic infections may affect HIV disease progression, perhaps by increasing HIV replication during the period of acute disease or by increasing cytokines that in turn have an impact on disease progression (Sarvolatz et al., 1996).

Respiratory symptoms are a frequent complaint in HIV-infected individuals. Pulmonary tuberculosis caused by *Mycobacterium tuberculosis* is the most common opportunistic infection in several developing countries (Kumarasamy et al., 1995). Tuberculosis is a frequent and treatable cause of morbidity and mortality in human immunodeficiency virus-infected patients. In HIV infected patients disseminated and extra pulmonary diseases are also common. TB can increase HIV replication either at local tissue sites or at a systemic level, there by accelerating the progression of HIV disease. TB associated increase of cell activation and cytokine production has been postulated as one of the possible mechanisms responsible for TB induced HIV replication and CD4 cell loss occurring during TB disease in HIV-infected individuals. Hence early diagnosis and appropriate treatment of tuberculosis is important to halt HIV disease progression.
India, a country despite with TB as an endemic disease, there are many reports on Tuberculosis in persons with HIV (Kumarasamy et al., 1996). Kumarasamy et al. (2001) observed pulmonary TB in 49% and extra pulmonary TB 11% in HIV infected patients. In our study on *Mycobacterium tuberculosis* we observed that the extra pulmonary TB was significantly associated with HIV/HBV (p=0.05) and HIV/HCV (p=0.013) than HIV alone infected cases where as the pulmonary TB (p=0.018) had significantly associated with HIV alone-infected cases than coinfected patients. The use of HAART has recently resulted in a dramatic decline of opportunistic infections (Palella et al., 1998). It remains to be seen if the incidence of PLD after successful reduction of HIV-RNA to undetectable levels using HAART will similarly show a decline.

Liver disease secondary to hepatitis C virus infection is a rising cause of morbidity and mortality among individuals who have been infected parenterally with human immunodeficiency virus such as injection drug users, hemophiliacs, and transfused patients. Until recently, the life expectancies of patients infected with HIV have been compromised by several opportunistic infection that develop when the CD4 lymphocyte count falls below a critical level. A change in recent years in the spectrum of illness associated with HIV infection has meant that certain previously unimportant diseases are now relevant (Soriano et al., 1995). Chronic liver disease, particularly chronic hepatitis C, is one of these conditions and is most frequently seen in countries where injection drug users (IVDUs) constitute a significant proportion of the HIV-infected population; chronic hepatitis C is certainly a growing cause of morbidity and mortality in the HIV coinfected groups (Soriano et al., 1995).

Oral Candidiasis is the most common oral manifestation in persons with HIV disease (Greenspan et al., 1990). Many oral lesions occur in the early course of
HIV disease and hence the presence of oral lesions may be early diagnostic indicator of immunodeficiency and HIV infection. Hence, early recognition, diagnosis and treatment of HIV-associated oral lesions may reduce morbidity. Previously Kumarasamy et al. (2001) observed 55.7% of oral candidiasis in HIV infected patients, in 2001 where as in 2003 our findings revealed that the oral candidiasis was significantly higher in HIV/HBV (p=0.001) and HIV/HCV (p=0.001) than HIV alone infected cases. In the most prevalent symptoms of lymphadenopathy (p=0.045) had associated significantly with HIV alone-infected cases than coinfected patients. The Pneumocystis carinii pneumonia is a common opportunistic disease that occurs almost exclusively in persons who have profound immunodeficiency (Frenkel et al., 1976). But in our observation we found two cases (3%) in HIV alone-infected cases and only one (3%) in HIV/HBV and not in HIV/HCV coinfected group.

Liver morphology in HCV infection features a high prevalence of chronic hepatitis with piece meal necrosis and or cirrhosis (Rugge et al., 1983 and Bach et al., 1992). Patients with HCV infection are sometimes found to be coinfected with HIV because epidemiologically the two infections overlap. HIV has no effective hepatotropism and liver lesions in AIDS are generally due to opportunistic infections or hepatotoxic factors, to which subjects at risk or contractive HCV and/or HIV infection are often exposed (Lebovics et al., 1985; Gorden et al., 1986 and Lebovics et al., 1988).

Generally the gastrointestinal and hepatobiliary disorders are among the most frequent complaints in patients with HIV disease. 50% to 93% of all patients with HIV disease had marked gastrointestinal symptoms during the course of their illness (Malenbranche et al., 1983). Diarrhea is the most common GI symptoms in persons with HIV. The prevalence of diarrhea ranges from 0.9% to 14% (Wilcox...
et al., 1996). In advanced HIV patients, the prevalence of diarrhoea is up to 50%. Very few reports are available on the pathogenic profile of chronic diarrhoea from India (Kumarasamy et al., 2000). Cryptosporidium is normally present in the gut of several Indians and can reactivate and cause diarrhea in the immunosuppressed. Counseling on boiling the water, commonly used for drinking, preparation of food and as an additive to fruit juices should be stressed to prevent diarrhoeal diseases. Kumarasamy et al. (2001) observed chronic diarrhea in 13.6% of patients. In our findings the cryptosporidial diarrhoea was higher in HIV/HBV (7%), HIV/HCV (9%) than HIV (3%) alone-infected cases.

HIV infection may be associated with a number of different cutaneous manifestations, some of which may be the presenting signs of the disease. Cutaneous manifestations of Cytomegalovirus (Lin et al., 1981), Herpes zoster, Herpes simplex (Seigal et al., 1981), have been documented in acquired immunodeficiency syndrome. In one study Kumarasamy et al. (2001) observed Herpes zoster in 11.2% of patients, but in our study group almost 19% of HIV-alone infected patients had Herpes zoster followed by 9% in HIV/HCV and 7% in HIV/HBV. The herpes simplex virus was observed only in the HIV/HBV coinfected group not in others.

Neurologic complications of HIV are common and not confined to opportunistic infections. Neurologic disease is the first manifestation of symptomatic HIV infection in roughly 10% to 20% of persons, whereas about 30% to 40% of patients with advanced HIV disease will have clinically evident neurologic dysfunction during the course of their illness (Levy et al., 1985). Cryptococcal meningitis, Toxoplasmosis, Varicella zoster, and CMV are the most common neuro-opportunistic infections. Few reports on neurological manifestations of HIV disease are available from India (Kumarasamy et al. 1995 and Wadia et al.,...
In our study the cryptococcal meningitis was higher in HIV/HBV (5%) than HIV (2%) group. The toxoplasmosis was observed in all three groups, but the incidence was higher in HCV coinfect ed group (9%) followed by HBV coinfect ed (3%) and HIV alone infected (2%) group. In one study Kumarasamy et al. (2001) observed 4.8% of the individuals had Cryptococcal meningitis and was the most common neuro-opportunistic infection and the toxoplasmosis was observed in 3.3% of patients, which is almost same to that of our study.

The improvement in AIDS mortality statistics reflects drug usage in Western Europe and the America. Opportunistic disease has been reversed and prevented. Health care costs have diminished. Many ill and disabled patients have returned to normal and functional life-styles. This dramatic impact does come with a cost- the expense, inconvenience, and toxicity of antiretroviral therapy. These costs and the benefits of treatment create a tension in the decision making process regarding when to initiate therapy (Palella et al., 1998). The clinical manifestations of HIV disease are primarily opportunistic consequences of the progressive destruction of the immune system by the persistent replication of HIV. Some manifestation including dementia, wasting, thrombocytopenia, and neuropathy can be the direct consequences of HIV infection and are often directly responsive to antiretroviral therapy. In our findings of overall opportunistic infections, hepatitis B and/or hepatitis C virus coinfect ed patients those who are taking ART are less likely to be infected by the HIV associated opportunistic infection compared to the patients not taking the ART.

Finally in our overall study we observed that most of the HIV associated opportunistic infections (Cryptosporidial diarrhoea, Cryptococcal meningitis, Extra pulmonary TB, Oral candidiasis and Toxoplasmosis) were significantly higher in coinfect ed cases than that of HIV alone-infected cases; But the analysis based on the
patient's ART status, the opportunistic infections can be controlled very effectively irrespective of associated hepatitis B & C virus coinfection. With improved combination of antiretroviral therapies now becoming possible leading to prolonged survival in HIV infected patients, the importance of developing the effective hepatitis treatment becomes paramount. However, the greater burden of these diseases falls mainly upon developing countries and economic constraints make access to many of the treatments unattainable.

6.2.4 Ultra sonogram findings of the liver in HIV, HBV and/or HCV coinfected patients with and without Antiretroviral therapy.

Liver failure has been reported as a cause of death in HIV-infected patients, particularly in patients with previous exposure to blood and blood products; it caused 7% of deaths in British HIV-infected patients with hemophilia and 4.8% of in-hospital mortality in Spanish HIV infected drug users (Soriano et al., 1999). The hepatomegaly, splenomegaly, transaminase elevations were commonly seen in HIV and hepatotrophic viruses coinfected patients. To assess the impact of hepatotrophic viruses coinfection in HIV infected cases the liver related clinical events were analysed individually. Liver related clinical events included the findings of USG or CT scan (e.g. Hepatomegaly, hepatosplenomegaly, mild hepatomegaly with ascites, moderate hepatomegaly, splenomegaly, and fatty liver). In our study the abnormal liver findings in HIV alone-infected patients were just 37% and the remaining 63% of them found to be normal liver. In the HIV/HBV coinfected cases almost 45% of them were found to be an abnormal findings of liver by USG study where as in the HIV/HCV coinfected group almost 70% of them were found to be an abnormal liver, it seems that the number of liver abnormality in HCV coinfected group were higher than that of HBV coinfected group. The over all liver abnormal findings, Analysis of the coinfected group with HIV alone-infected cases, revealed that for
overall liver findings, the difference was not statistically significant (HIV vs. HIV/HBV $p=0.492$; HIV vs. HIV/HCV $p=0.080$; and HIV/HBV vs. HIV/HCV $p=0.281$). Though its not statistically significant the over all analysis reveals that HCV associated liver disease progression is seen in the HIV infected group compare to the HBV related liver complications in HIV coinfected group. In the USG/CT scan study of the liver in the HIV alone-infected group the response to the drug (ART) shown statistically significant ($p=0.005$) variations in the abnormal and normal liver findings. In the HIV/HBV and HIV/HCV coinfected group the USG/CT scan level liver findings difference was not seen in between the patients those who are taking the ART and naïve. It clearly shows that the hepatitis B virus (HBV) and hepatitis C virus (HCV) influences the ART response in HIV infected patients.

HIV infection modifies the natural history of chronic parenterally acquired hepatitis C with an unusually rapid progression to cirrhosis. HIV related immunodeficiency may be a determinant of higher hepatitis C viremia levels and more severe liver damage (Soto et al., 1997). The major consequence of chronic HCV infection is progression to hepatic cirrhosis and its associated clinical complications, namely, ascites, jaundice, variceal bleeding and encephalopathy, and hepatocellular carcinoma (HCC). In person not infected with HIV, HCV related cirrhosis develops in 20%-30% (Di Bisceglie et al., 1991) within 2-3 decades after exposure. Some studies of hemophiliacs who developed progressive liver disease (PLD), PLD occurred more frequently and earlier in HIV/HCV coinfected hemophiliacs than in hemophiliacs infected with HCV alone (Eyster et al., 1993). Patients with low CD4 cell counts had higher risks, of developing PLD; however, progression to AIDS was not associated with a higher risk of developing PLD. Like other viruses caring opportunistic infections, such as cytomegalovirus or herpes
simplex virus, HCV may also be viewed as a relative opportunistic pathogen leading to the risk of PLD in coinfected persons than in persons infected with HCV alone (95% CI 2.2-25.5), (Fletcher et al., 1983).

Some studies focusing on the histological activity of CHC in IVDU have suggested that hepatic disease might be milder in these individuals than in those with post transfusion CHC. Although the factors influencing the histological activity remains to be established, it has been pointed out that infection with certain HCV genotypes could play a role in the severity of liver damage in patients with chronic liver disease related to HCV (Pozzato et al., 1994). On the other hand, human immunodeficiency virus (HIV) infection is prevalent among IVDUs in most countries, and several reports have pointed out that HIV immunosuppression could influence the natural course of HCV infection, causing more rapid progression to end stage liver disease (Martin et al., 1989 and Eyster et al., 1993).

Histological liver damage of CHC as scored by Knodell’s index was significantly higher in patients carrying HCV subtype 1b than in those infected by other variants. On the other hand the significant correlation between HCV-non 1b patients and sustained response to interferon reported elsewhere (Yoshioka et al., 1992) could be related to their low HAI. An increase in survival of HIV infected persons related to active antiretroviral therapies (Gulick et al., 1997) highlights the problem of chronic hepatitis C in HIV coinfected patients. HCV related liver disease may be more severe in HIV infected people than in non-HIV infected individuals (Eyster et al., 1993 and Soto et al., 1997). The prevalence of cirrhosis may be 3-fold higher in HIV-HCV coinfected patients than in HIV-negative HCV-infected patients (Soto et al., 1997).
The liver damage in patients with CHC is directly influenced by HCV subtypes. Infection with HCV subtype 1b is closely associated with more severe forms of liver pathology mainly higher degrees of piecemeal necrosis and fibrosis. Recently, some authors have reported on the probability of patients coinfected with HIV and HCV having faster progression of hepatitis C, including a rapid evolution to decompensated cirrhosis (Martin et al., 1989) or hepatic failure (Eyster et al., 1993), or a lower interval from estimated time of HCV infection to cirrhosis in patients with parenterally acquired hepatitis C (median interval of 6.9 years in HIV positive patients vs 23.2 years in HIV negative ones. p<0.001) (Soto et al., 1997). In contrast, Wright et al. (1994) found in a retrospective study, including predominantly homosexual HIV positive patients, that the presence of HCV infection did not adversely influence their survival (Wright et al., 1994). Chronic hepatitis C was reported to be more severe in patients with HIV infection than in those without this disease; it can lead to cirrhosis with fatal complication (Martin et al., 1989). Chronic hepatitis C patients with HIV infection, the response and tolerance of recombinant interferon- α were not different from those usually observed in patients with chronic hepatitis C infection without HIV infections (Boyer et al., 1992).

In our analysis the USG/CT scan studies of the liver in HIV alone infected cases shows that only 33%of patients had normal findings and almost 67% of the naïve cases developed the initial stage of liver abnormalities where as the patients taking ART the liver abnormalities were 23% and almost 77% of the patients showed normal liver findings, it reveals that the ART plays very important role compare to naïve (without ART) in the HIV alone infected cases. In the HIV/HBV coinfected group the liver abnormalities was only 40% in the naïve compared to 67% in the HIV alone-infected group. It shows that the USG findings
of liver abnormalities is low in coinfected ART naïve group, but in patients taking ART in the coinfected group almost 54% of patients had abnormal findings, which is higher than that of HIV alone infected group (23%) taking ART. In the HIV/HCV group 66.7% of patients had abnormal liver findings in the ART group, which is higher than that of HBV, coinfected group. Hence the USG/CT scan findings of liver abnormalities in patients taking ART indicate that ART induced the immunocompetent CD4 cells and this might be the reason for the abnormalities of the liver in coinfected group. In the coinfected cases the difference in the serological and molecular profiles of HBV and HCV was not seen in the ART and naïve group. In our study, cases with the absence of liver biopsy would be difficult to comment on the presence of chronic liver disease in our patients.

Eyster et al. (1993) observed that hepatomegaly, splenomegaly, and transaminase elevation in transfused subjects who were infected with HCV and HIV. Out of 65 patients with hepatomegaly, 74% were coinfected with HIV and HCV. In the 110 subjects without hepatomegaly 35% had no infection, 31% had single infection with HCV, and 33% had coinfection with HCV and HIV. In the 57 patients with splenomegaly 82% were coinfected whereas 115 without splenomegaly 38% had no infection, 31% were infected with HCV only and 31% were coinfected. Out of 82 subjects with transaminase elevation, 38% had single infection with HCV and 60% were coinfected. Liver failure occurs more frequently in coinfected individuals than in those infected with HCV alone. Both AIDS and liver failure are normally associated with lymphocytopenia and immune deficiency with CD4 count less than 100 cells/μl (Eyster et al., 1993).

Anecdotal evidence suggests that chronic viral hepatitis may be associated with increased risk of antiretroviral drug associated hepatotoxicity (Rosado et al., 1998 and Markowitz et al., 1998). However, the actual incidence of
drug-induced hepatotoxicity and role of chronic viral hepatitis are poorly understood since anecdotal reports omit the number of exposed persons and may focus attention on exceptional cases or high-risk populations. In one study Sulkowski et al. (2000) observed that the risk of chronic hepatitis B & C virus infection. Similarly, Rodriguez-Rosado et al. (1998) found that chronic HCV infection was associated with 2.8-fold greater risk of hepatotoxicity with use of highly active antiretroviral therapy. The data of Sulkowski et al. (2002) suggest that ART should not be withheld from persons infected with HIV with chronic viral hepatitis, and may also support the practice of continuing ART in the presence of mild-to-moderate hepatic aminotransferase elevations with careful clinical monitoring.

The mechanism of antiretroviral-associated hepatotoxicity in patients with or without chronic viral hepatitis is not known, but is likely to be multifactorial. Sulkowski et al., (2002) found that CD4 cell recovery was independently associated with severe hepatotoxicity, suggesting that hepatic injury may be immune mediated in some patients. However, although some studies have suggested that hepatic injury may be caused by enhanced HCV replication and cytotoxic T cell activity during HAART associated immune reconstitution, other studies have failed to confirm this hypothesis (Rutschmann et al., 1998). In addition, CD4 cell increase may merely be a marker of greater adherence to HAART, which may also increase the risk of observing hepatotoxicity. A recent study showed that patients coinfected with HIV and HCV type-1 experienced a more rapid progression to AIDS or death than did those infected with HIV and other types of HCV, independent of age and changes in CD4 T-cell count during the follow-up period (Sabin et al., 1997) and that result suggests an association between HCV (HCV genotype) and progression of HIV disease.
6.2.5 CD4 and CD8 counts in HIV, HBV and/or HCV coinfectected patients

In HIV infected individuals, the rate of CD4 T-cell loss exceeds the rate at which CD4 T cells are produced through thymic differentiation and clonal expansion of peripheral CD4 T cells. CD4 T-cell may be lost through a number of potential mechanisms, some operative for non-infected cells. In addition to the quantitative depletion of CD4 T cells that occurs during the course HIV-1 infection, there are qualitative defects in the function of the surviving CD4 T cells. The CD4 cell count is the most useful marker for predicting the immediate risk of developing a particular opportunistic infection. Such complications are rare in patients with CD4 counts above 500 cells/mm³. As the CD4 counts drops below 500 cells/mm³, patients may begin to experience different types of opportunistic infections. The pathobiology of chronic HBV infection is complex. A number of factors may have observed existing relationship and hepatic inflammation, cellular immune function and HBV replication. These may be a significant difference between circulating lymphocytes and those present in the liver. There may be differences in the manner of expression of viral antigens on the membrane of the hepatocyte that influence the inflammatory reaction to HBV (Chu et al., 1986).

HCV infection is controlled by the cytotoxic lymphocytes (CTL), which eliminate the infected hepatocytes, and by cytokines produced by the T cells, which directly inhibit viral replication (Battegay et al., 1996). The immune response against viral infection appears in two ways: the CD4 Th1 cells produce cytokines which activate the CTL (CD8) response, whereas the CD4 Th2 cells induce the production of specific antibodies against HCV. It has been suggested that a poor response of the CD4 Th1 subset might be related to the chronicity of HCV infection. This defective response might induce a change in the CTL that makes it more difficult to eliminate HCV. This possibility could explain the higher activity
(virulence) of HCV infection in HIV-infected patients, whose CD4 cells were defective in function and number. Puoti et al. (2002) observed that the ALT increase was independent of variations of peripheral CD4 and CD8 positive subpopulations. Base line HCV-RNA levels were significantly lower in patients showing a 2-fold ALT increase. Low levels of HCV viremia in HIV infected patients could be explained by the presence of an efficient control of HCV replication by CD8 and CD4 anti-HCV specific lymphocytes. The ability to rapidly mount an effective, specific intrahepatic immune response might explain the increase in liver cell necrosis that occurred promptly and regardless of variations in peripheral CD4 and CD8 cell counts. Finally the author concluded that the HCV-RNA increase occurs in most HCV-HIV coinfected patients during combination ART: thus increase is independent of treatment regimen, liver cell necrosis and variations in immunologic parameters, and it appears to be related to an exponential decrease in HIV replication. This HCV-RNA increase might be associated with liver cell necrosis in patients with lower baseline HCV replication and is probably related to a pre-existing effective immune response to HCV.

Rugge et al. (1994) evaluated and compared the morphological lesions of the liver in HCV/HIV positive patients, from a group of patients not infected with HIV. They observed that liver damage was significantly more severely HCV positive/ HIV negative patients and in HCV/HIV positive when the CD4 cells count was higher than 400/mm$^3$, whereas a less severe liver histology in HCV/HIV coinfected patients correlated with the drop in the CD4 lymphocyte cell count. It is still not clear whether the influence of HIV in the histology of hepatitis C is only due to the decrease in CD4 circulating lymphocytes. Evidence has been provided of HIV infection in sinusoidal Kupffer's cells and endothelial cells (Houset et al., 1990), which possess CD4 molecules. Previous studies have suggested that, in viral
hepatitis, endothelial cells activated by macrophages express adhesion molecules (Volpes et al., 1990), which play a role in retaining lymphocytes in the liver sinusoid initially and then migration through the endothelium. These steps are crucial before any interaction occur between lymphocytes and the hepatocytes (Rieder et al., 1992). That liver disease is less severe in immunodepressed HCV/HIV coinfected patients may also be explained by a defective local immune response due to HIV infection of sinusoidal cells.

It has been proposed that patients who are HIV positive and have CD4 counts below 400 cells/ul show significantly less portal inflammation and piecemeal necrosis (Guido et al., 1994), which suggest that progression of liver lesions in patients with hepatitis C may depend on immune mediated mechanisms (Guido et al., 1994). In contrast Rockstroh et al., (1996) datas did not support that hypothesis because they noted a high frequency of cirrhosis in autopsy in the hemophiliacs infected with HIV and HCV in comparison to the hemophiliacs who were HIV negative but HCV positive and to the AIDS patients, who were HCV negative. Finally the authors suggested that concomitant HCV Infection in hemophiliac infected with HIV is associated with significant morbidity and mortality. Failing cellular immune function is apparently a strong risk factor for that complication of AIDS, and severe cholestasis in those patients may indicate that from deteriorating liver function. Because liver failure occurs only after long periods of HIV/HCV infection, complications is likely to increase in coming years. Benhamou et al. (1999) concluded that HIV seropositivity accelerates fibrosis progression related to chronic hepatitis C, HIV infection, alcohol consumption of more than 50g/day, CD4 lymphocyte count less than 200 cells/ul, and age of more than 25 years at HCV infection are associated with an increased rate of liver fibrosis.
progression in HCV-coinfected patients. These factors should be considered in anti-
HCV therapeutic strategies.

Our study reveals that the overall CD4 and CD8 counts in the HIV alone-
infected cases were higher than that of coinfected patients. The difference in total
CD4 and CD8 counts were statistically not significant. The over all depletion of the
CD4 counts, especially in the HIV/HCV coinfected patients than HIV/HBV
concludes that the impact of HCV coinfection was much severe than HBV
coinfection, and this may lead to faster progression of HIV associated opportunistic
infection. The coinfection by HCV seems to be a detrimental prognosis cofactor for
HIV disease, accelerating the drop of CD4 cells. A strong negative correlation
between HCV-RNA levels and CD4 counts, suggesting that HIV induced immune
deficiency may allow increased HCV replication (Eyster et al., 1994). Very high
viral loads of $2 \times 10^7$ to $3 \times 10^9$/ml were seen only in HIV positive individuals. Thus,
HCV may be reactivated or inefficiently cleared in the immune deficient host.
Alternatively, immune deficient individual may be less able to respond to the
emergence of certain HCV variants, or may be more susceptible to reinfection from
plasma concentrates certain virus below the level of detection in hemophilic
patients. There is a good reason to suspect that HIV positive hemophiliacs are at
higher risk for the development of HCV related liver failure as well for the
transmission of HCV to their sexual partners (Eyster et al., 1991).

The outcome of viral infection represents a balance between viral factors
(i.e., replication rate, mutation, dose, and cytopathic effects) and the host immune
system. These factors effect both viral eradication and tissue damage. The cellular
immune response, based on the interplay between CD4 helper cells and CD8 cytotoxic T cells is important in viral clearance, whereas humoral immunity is generally more important in the prevention of secondary infection. Viral infection is controlled by both cytotoxic T cells, which lyse virus-infected cells, and by cytokines produced by T cells, which directly inhibit viral replication. CD4 cells regulate the immune response through two pathways. Th1 CD4 cells produce cytokines which activate the cytotoxic T cells response, whereas Th2 CD4 cells are involved in antibody production. It is expected that immunosuppression affects outcome from viral infection because it causes an increase in the viral load and also because it impairs CD4 Th1/cytotoxic (CD8) T cell responses (Jane Collier et al., 1998). Hepatic fibrosis is a wound healing response to ongoing liver injury that leads to architectural liver changes and culminate in a cirrhosis and, eventually, liver failure. Two hypothesis can be advanced to explain the relationship between CD4 lymphocyte depletion and an accelerated evolution of liver fibrosis. First, in HIV-HCV coinfected patients, CD4 cell counts may be negatively related to HCV-RNA levels. Thus, increased HCV viremia may accelerate fibrosis. The second hypothesis invokes an alteration of cytokine patterns associated with CD4 lymphocyte depletion. Such an attention was recently shown for anti-HCV specific T cells in HIV seropositive patients. In an experimental mouse model, there is a relationship between the host’s immune phenotype and the nature of fibrotic response. In one study Puoti et al. (2000) concluded that independent association between HIV-infected CD4 lymphocyte depletion and increased rate of fibrous septa formation in patients with HCV infection, and they have suggested that early combination ART
might interrupt the vicious cycle between CD4 cell depletion and accelerated progression of liver disease in patients with HIV-HCV coinfection.

In our study, the overall CD8 cells count in the HIV/HCV coinfected patients also lower than HIV and HIV/HBV coinfected patients, but the difference was not statistically significant. The overall HIV-RNA in HIV/HBV coinfected patients was 276312.9 copies / ml, which is much higher than that of HIV alone-infected group, again the difference was not significant (p=0.22). The overall mean of HIV-RNA load in HIV/HCV coinfected patients was lower than HIV alone-infected patients. It shows the possibility that the coinfected patients may influence the HIV diseased condition. An attempt was made to analyse, the difference in CD4 & CD8 cell counts in HBV-DNA positive and HBV-DNA negative cases of HIV/HBV coinfected patients. However, no such difference was observed in that group, nor in HCV-RNA positive and HCV-RNA negative patients. So we conclude that the viremic status of the hepatitis B and hepatitis C does not influence the CD4 and CD8 depletion.

6.3 STUDIES ON INTERACTION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) AND ASYMPTOMATIC CHRONIC HEPATITIS B VIRUS (HBV) INFECTED INDIVIDUALS

Human immunodeficiency virus (HIV) infection appears to influence the natural history of infections with certain hepatitis viruses. Interaction between the HIV and concurrent infections with hepatitis viruses may alter the natural history and treatment response of both diseases (Brendon et al., 1998). There is high degree of epidemiological similarity between the hepatitis B virus (HBV) and HIV as
regard to high risk groups, routes of transmission and the presence of virus in the body fluids (Rustgi et al., 1984).

Although India is classified under intermediate prevalence zone, variations in the HBV infection rates among general population in different states of India, as evidenced mostly by screening of voluntary blood donors is observed. Our own (Hari, 2001) previous study revealed that the HBsAg positivity rate was 5.7% (95% CI 4.65- 6.75%). A review by Tandon et al. (1996) suggested that in India HBV infection is established in early childhood and horizontal means of transmission maintained the carrier pool.

The first HIV positive result was detected in 1979 in male homosexual. Prevalence of HIV in the HBV homosexual carriers ranged from 8% in 1979 to 49% in 1983. The prevalence was significantly lower in patients with acute hepatitis B than in patients with chronic hepatitis B, and in nonhomosexual subjects compared with homosexual subjects.

Recently in one study on seroprevalence of anti-HCV and hepatitis B surface antigen in HIV infected patients, 25.8% and 5.6% were positive for HBV and HCV respectively (Tankhiwale et al., 2003). In another study in India, Mathur et al., (2002) reported 6.1% HIV positivity in HBsAg positive cases.

In our study out of 155 chronic HBV carrier cases, 3 (1.9%) were anti-HIV positive and all the 3 were male gender only. The HIV coinfection in our study group is of very low percentage when compared to the previous study on coinfection in India. The HIV & HBV coinfection among Indian patients has been variably reported as 2.5% to 81% (Lakshmi et al., 1998 and Rathi et al., 1997). Since the modes of transmission of HIV and hepatitis B virus and the risk groups affected by these two agents are so similar, a hepatitis clinic population provides a useful group
to study the natural history of the HIV epidemic. An analysis of the mode of acquiring HBV in this group of asymptomatic carriers had revealed play injury 16%, hospitalization 13%, previous h/o jaundice 17%, surgery 8%, unsterile needle 19%, blood transfusion 1% and 26% of HBV carriers could not provide any traceable history. In the 3 HIV coinfectected patients two had previous history of surgery and one with the history of hospitalization but none of our study group patients had previous history of sexual promiscuity. The prevalence of HBV coinfection with HIV varies in geographical regions of the world depending on the prevalent mode of transmission of disease. Though sexual transmission of hepatitis B virus is known to be more efficient by homosexual contact and the infection is transmitted by heterosexual contact fairly efficiently (Dietzman et al., 1977). In India heterosexual transmission accounts for 75% of the HIV infections (Bollinger et al., 1995). This could be the possible explanation of the low prevalence of HIV coinfection in our study group compared to the other studies in India.

Krogsgaard et al. (1987) observed hepatitis B virus DNA clearance rates in the two groups were significantly different (p<0.05). They observed that disease activity, as determined by transaminase levels, was significantly lower in HTLV-III infected individuals as compared to individuals without HTLV-III infection (p<0.05). In contrast, our findings revealed that the HIV/HBV coinfectected patient’s ALT was elevated (mean ALT=72 IU) compared to the individuals without HIV coinfection (mean ALT=53.7 IU). This finding suggests that the coinfectected patients are likely to develop liver complications compared to the chronic HBV alone infected group.

McDonald et al., (1987) observed HIV positive HBV carrier had many more HBe and anti-HBe positive hepatocyte nuclei than anti-HIV negative carriers (p<0.003 and p<0.002 respectively), and HBV-DNA levels were slightly, but not
significantly, increased in the positive subjects. They have concluded that male homosexual HBV carriers, positive for anti-HIV, may be immunosuppressed before there are clinical signs of immunodeficiency, and this allows an increased level of replication of at least one other virus (HBV).

In another study McDonald et al. (1987) observed that the percentage reduction of HBV-DNA was significantly less in the anti-HTLV-III positive group in comparison to the anti-HTLV-III negative group at 1 and 4 months of treatment and at 3 months after the end of treatment (p<0.05). Those patients who were younger (33 vs 42 years, p<0.002) had lower mean baseline AST values (42 vs. 80 IU per liter, p<0.02) and tended to have milder histological disease.

Chronic hepatitis B virus (HBV) infection is a major health problem throughout Asia and Africa, and among "risk groups" in the developed countries. HBsAg positive liver disease is associated with an increased risk of cirrhosis (Chu et al., 1985) and hepatocellular carcinoma. The first HIV positive tests were noted in homosexuals in 1980. The prevalence in acute HBV homosexuals, ranged from 12% in 1981 to 33% in 1983 (De Cock et al., 1987).

Earlier seroepidemiologic studies have shown that about half of all homosexual men in the United States show some marker of prior infection with hepatitis B virus (Szmuness et al., 1975) while almost all persons who have abused intravenous drugs for five years have been infected (Lettau et al., 1985). Interacting factors for the development of chronicity in hepatitis B infection include infection in life, male sex, and asymptomatic acute infection (McMahon et al., 1986). One study showed that when HIV-1 infection precedes HBV infection, the risk of becoming an HBV carrier is increased three fold (Taylor et al., 1998).
In our 3 HIV coinfect ed cases, two were HBeAg (67%) positive and also HBV-DNA positive. There is a significant difference in the HBeAg positivity and anti-HBe positivity pattern in the HIV positive and HIV negative chronic HBV infected cases (p=0.0043) in the present study.

Among the remaining 152 HBsAg positive cases without HIV coinfection, 124 cases were tested, in which 11% were HBeAg positive and 88% were anti-HBe positive. The HBV-DNA positivity in HBe positive and anti-HBe positive cases differed significantly (p=0.001) as observed by many investigators.

Many patients with chronic HBV infection were also infected with the human immunodeficiency virus (HIV). The impact of these infections on liver histology and HBV replication may be important in prognostic and therapeutic terms. In addition, the influence of immune deficiency on HBV infections can be studied in HIV-positive subjects. It has been suggested that HIV infection enhances HBV replication but ameliorates the associated liver injury (Rustgi et al., 1984; Gorden et al., 1986 and Perillo et al., 1986). However, other studies have led to different conclusions, and all have shortcomings because of the heterogeneity or small number of the patients (Rustgi et al., 1984; Gorden et al., 1986; Lebovics et al., 1985 and Rector et al., 1988). Housset et al. (1992) retrospectively analysed a large number of chronic HBV carriers in an attempt to clarify the interactions between HIV and HBV infections; and they also indicated that the coexistence of HIV infection with HBV may accelerate the onset of severe liver disease, and that the course of chronic HBV infection is influenced both by the presence of other chronic viral infections and by the risks factors for exposure to HBV.

In one study, Koblin et al. (1992) observed HBV-DNA and HBeAg were inversely related to duration of hepatitis B virus infection (p<0.001) and they have
concluded that hepatitis B virus replication among chronic carriers may be a function of duration of hepatitis B virus infection rather than that of an effect of human immunodeficiency virus type-1.

Mai et al. (1996) observed that the prevalence of HBeAg was significantly higher in HIV/HBV coinfected group (85.7%) than HIV antibody negative group (65%). There was a significantly higher mortality rate observed in the HIV antibody positive group versus the HIV negative group (p<0.05) and 75% of deaths in the HIV antibody positive group were due to an AIDS defining illness. Hence, HIV coinfection did not appear to alter the mortality rate from liver disease in these hepatitis B carriers. Finally they have concluded that HIV-infected patients, death from AIDS predominated and death from liver diseases was low. Hence, the main target for therapy should be HIV rather than HBV.

Colin et al. (1999) observed that homosexual men with chronic hepatitis B and HIV infection is associated with a higher level of HBV replication and a higher risk for cirrhosis without increased liver necroinflammatory process.

In our study, when we analysed the 3 HIV coinfected chronic HBV patients individually, two of them showed positivity for HBV-DNA and they showed elevated ALT (72 IU and 82 IU) than HBV-DNA negative HIV/HBV coinfected case (ALT=56 IU). In the ultra sonogram study of the two HBV-DNA positive patients one had moderate hepatomegaly and the remainig one patient had normal liver, whereas, the one HBV-DNA negative case showed the hepatomegaly with portal hyper tension (PHT).

The outcome of chronic HBV infection depends on a tolerance between the virus, which is not thought to be directly cytopathic, and the host's immunological response to it. This theory supported by observations of the natural
history of chronic HBV infection (Chu et al., 1985), where during the high replicative phase there is only minor liver damage and, at the stage when HBV-DNA levels starts falling, there is an increase in disease activity and a rise in the transaminase, presumably the result of cell mediated attack of HBV infected hepatocytes. But in the absence of liver biopsy it would be difficult to comment on the presence of chronic liver disease in our patients. In one study Housset et al. (1992) concluded that chronic HBV infection could be associated with severe liver damage in HIV-positive drug abusers and homosexuals. HIV infection does not seem to attenuate and may even worsen HBV chronic liver damage and they also emphasized the important role of the risk factor for exposure to HBV and of chronic alcohol consumption in the analysis of HBV-related liver diseases.

In conclusion although India is classified under intermediate prevalence zone, variations in the HBV infection rates among general population in different states of India is observable. Chronic hepatitis B virus (HBV) infection is a major health problem throughout Asia and Africa, and among “risk groups” in the developed countries. The coinfection rate is also likely to vary with the prevalence rate patterns of these two infections (HIV and HBV) in the general population. The prevalence of HBV carriers in India is 2-7% (Nishioka et al., 1975) and that of HIV infection is 29.4/1000-screened populations (NACO, India). There was significant difference in transaminase levels of HIV/HBV coinfected and HIV alone infected patients indicating associated liver disease in such coinfected cases. With increasing incidence of HIV infection in our country and longer life expectancy in these coinfected patients, HBV associated liver disease is likely to emerge as a major chronic complications requiring careful public health care.
6.4 STUDIES ON HUMAN IMMUNODEFICIENCY VIRUS (HIV) COINFECTED CHRONIC LIVER DISEASE (CLD) PATIENTS

One common problem in chronic liver disease is the high prevalence of hepatitis B virus (HBV), which is on top of causes of chronic hepatitis, liver cirrhosis and liver cancer (Suzuki & Woodfield, 1994). HCV ranks significantly behind HBV in the pathogenesis of CLD in the US, erstwhile USSR and most Asian countries, except Japan where HCV is the leading cause (Suzuki et al., 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 HBV positivity between 35-65% and a HCV positivity of 12-35%. In one of our previous study, the HBV positivity was recorded in 38.7% of the cases and HCV positivity was seen in 18.2% of the cases, which is very similar to that observed in the above reports.

Many Indian reports have implicated HBV as the major etiologic agent of CLD followed by HCV (Mehta et al., 1992 and Tandon et al., 1994). The reported HBV positivity in these studies was between 40-70% and HCV positivity was recorded in 16-26% of the CLD cases. However, in one study (Irshad et al., 1994), a high HCV positivity of 48.5% was observed. Of course the patient population in this study consisted of only CAH cases. In our previous HIVseroprevalence study on HCC & CLD cases revealed that none of the HCC cases had HIV infection; however, 3 out of 95 cirrhosis of liver cases had HIV seropositivity and all the 3 in virus positive group (1 out of 30 HBV positive cirrhosis and 2 out of 35 HCV
positive cirrhosis of liver cases). In our study of 192 CLD cases, 24% was HBV infected (95% CI 18-30) 13% were HCV infected (95% CI 8-18) and the remaining 63% CLD cases were non-B & non-C category (95% CI 56-70%).

The overall HBsAg antigenemia among liver disease patients was 24%, which is in concordance with findings of other workers in India, with HBsAg detection rate varying between 12.2%-57% (Dharmadhikari et al., 1990). In our study, the prevalence of HIV infection in HBV infected CLD patients was found to be 6.3% compared to that of coinfection rate of 28% to 85% reported by other workers (Dhanvijay et al., 1999). Chronic carriage of HBV (without HIV coinfection) has been reported to occur in 6% cases of liver disease and is found to increase to approximately 20% in patients who are infected with HIV prior to HBV exposure (Bodsworth et al., 1991). In our study, one patient (4.1%) was associated with overall 24 HCV infected CLD cases and the remaining 2 cases (1.65%) were associated with non-B & non-C CLD group.

In the present study, of the 3 HIV/HBV coinfect ed CLD cases, 2 (67%) are HBeAg positive and 1 (33%) was anti-HBe positive. In the remaining 44 HBV alone infected CLD cases without HIV coinfection, 23% had HBeAg positivity and 77% were anti-HBe positive. In the coinfect ed patients, HBV-DNA positivity was significantly (p=0.005) higher than HBV alone infected group. Bodsworth et al., (1991) reported that the HIV superinfection leads to increase in HBV replication but liver inflammation lessens and transaminase values decreases with the histological evidence of a less severe liver disease. Despite this trend towards a milder disease, acute asymptomatic recurrent hepatitis B has been reported, with abrupt onset of symptoms and elevated serum HBV-DNA levels in patients with HBV and HIV coinfection (Bodsworth et al., 1991).
The pathogenesis of hepatocellular damage by HCV is poorly understood. As with chronic hepatitis B infection, evidence is emerging that liver damage may be mediated by the immune reaction to infected hepatocytes, rather than by the virus itself (Koziel et al., 1992). However, the demonstration of HCV antigen in liver biopsies and the correlation between levels of viremia and degree of lobular inflammation suggests that HCV may be directly cytopathic to liver cells (Lau et al., 1993). In one study Elaine Eyster et al. (1993) observed that liver failure occurs more frequently in HCV seropositive hemophiliacs who are HIV seropositive than in those who are HIV seronegative. Those with lower CD4 lymphocyte counts or lymphocytopenia have an increased risk of liver failure. These findings suggest that HIV or its associated immune deficiency state may accelerate the development of liver failure, perhaps by enhancing HCV replication. They have concluded that HCV-RNA levels are significantly higher in HIV positive than in HIV negative multitransfused hemophiliacs. HCV load increases over time, is enhanced by HIV, and further increases as immune deficiency progresses. HCV-RNA levels are strongly associated with AST levels.

In our study the positivity pattern of HCV-RNA revealed that 73% RNA positivity rate was observed in HIV/HCV coinfected cases, while only 43% HCV-RNA positivity was seen in CLD cases infected with HCV only.

In the HIV infected CLD cases, the transaminase levels were not significantly increased (ALT & AST) compared to overall CLD cases without HIV association in our study. But the HIV infected CLD cases transaminase levels were significantly elevated than HBV alone infected CLD (p=0.05) cases. It clearly shows that HIV could influence the functional status of the liver in the CLD patients. In our study the overall transaminase analysis the non-B & non-C group of CLD patients showed elevated AST and ALT than other groups. The ALT was significantly higher
(p=0.005) in non-B & non-C CLD than HIV coinfected CLD and HBV alone infected CLD cases. The evaluation of transaminases in nonB & nonC CLD groups could be due to other factors including presence of aflatoxin, alcohol intake and immunological disorders.

Among the overall 6 HIV infected CLD cases, one patient was coinfected with HCV with elevated liver transaminase enzymes (ALT=128 IU, AST=124 IU). In the 3 HIV/HBV coinfected CLD cases, 2 were HBV-DNA positive of which one patient showed higher transaminase (ALT=122 IU, AST=98 IU) which was diagnosed as a liver cirrhosis by biopsy and one HBV-DNA negative patient enzyme was also elevated. Among the two HIV associated nonB, nonC CLD cases studied, one patient had higher ALT (150 IU) & AST (177 IU) was diagnosed as a CAH by liver biopsy.

Since 1996, highly active antiretroviral therapy (HAART) has dramatically improved survival rates among HIV-infected patients (Mocroft et al., 1998), but little information is available on the prognosis of HCV coinfection. Many studies, the correlation with immunosuppression, chronic hepatitis C has been reported to be more severe in HIV infected patients, which leads to higher levels of HCV viremia, more extensive hepatic damage, and faster progression to cirrhosis (Martin et al., 1989; Eyster et al., 1993 and Soto et al., 1997). However very few data have been published on the impact of chronic HCV infection on mortality among HIV infected patients. In one study Cacoub et al. (2001) carried out retrospective multicenter surveys in France during 1995 and 1997 and included 17,487 and 26,497 HIV infected patients respectively, revealed that the annual death rate because of HCV related cirrhosis or hepatocellular carcinoma is very low (0.06% and 0.07% respectively). In contrast with dramatic decrease in AIDS related
annual mortality rates from 7.4 in 1995 to 1.7\% in 1997, following the introduction of HAART (used since 1996), the HCV related mortality rate was not modified.

6.5 HEPATITIS B VIRUS (HBV) PRECORE /CORE MUTANTS STUDY

This study examined 9 Indian HBV infected individuals presenting with different clinical manifestations for possible association with Pre-C/BCP mutants. Nucleotide substitution in viral genomes can have several effects, including evasion of vaccine-induced or natural immunity, drug resistance, changes in pathogenicity, alterations in tissue or species tropism, and viral persistence. While RNA viruses exhibit closely related but non-identical variants (genotypes) and (Domingo et al., 1978), DNA viruses are less variable. This variability is due to inherent error-prone replication that results in 1 in 1000 to 1 in 100 000 point replacements, deletions, or insertion per nucleotide and round of copying (Domingo et al., 1978). These errors in viral genomes are compounded by a lack of proof reading repair-enzyme activities, in particular with reverse transcriptase, which would keep cellular DNA mutation rates below 1 in $10^8$.

HBV replicates via an intermediate RNA stage with the use of reverse transcriptase; it might therefore be expected to have a high mutation rate. But there are constraints on the ability of HBV to accept mutation without becoming non-viable. The genome is only about 3200 bases long, the smallest of any DNA virus that infect man, and all the genetic material codes for proteins with the regulatory elements being found within these coding regions. Multiple strains of HBV are found in chronic infection, implying that evolution occurs within patients.

In populations worldwide, variants of hepatitis B virus (HBV) are selected after seroconversion to antibody to HB e antigen (anti-HBe). Although the
variants were first described in patients with severe disease (Carman et al., 1989, Brunetto et al., 1990), there is some dispute regarding their degree of pathogenic potential. It appears that in patients with both progressive hepatitis and quiescent liver pathology, these variants are selected with equal efficiency (Tur-Kaspa et al., 1992). The core gene of HBV consists of two regions, the 29 aminoacid (aa) precore and 183 aa core each with an in-frame translational start codon (TAG). Thus two proteins can be produced: HBe antigen (HBeAg) is translated from the first (ATG), and hepatitis core antigen (HbcAg; nucleocapsid protein) is translated from the second. Hepatitis B e antigen (HBeAg), which is found free in serum but is not a part of the virion, is a marker of hepatitis B virion (HBV) replication and infectivity. It is believed to be a target for cytotoxic T cells and is found on the surface of vaccinia-recombinant transfected cells (Schlicht et al., 1989), making it a potential target for humoral immunity. HBeAg is derived by proteolysis of the translation product of the entire precore/core open-reading frame (Ou et al., 1986).

The most frequently observed precore mutation is a G to A transition at nucleotide 1896. This substitution introduces a translation stop codon (TAG) in the distal precore gene and prevents expression of the distal precore gene and prevents expression of the precore/core fusion protein that functions as a precursor of HBeAg (Careman et al., 1989). Less common precore mutations resulting in HBeAg negativity include initiates codon mutations (at position 1814 or 1815), a nonsense mutation at 1874, a missense mutation at 1862, and frameshift mutations (Kramvis et al., 1997). The serum of patients with these mutations does not contain HBeAg and hepatocytes harboring this mutant virus do not present HBeAg on their surface. Because HBeAg is an important immunological target, these cells escape killing mediated by the host's immune system.
The occurrence of precore/core mutants has been reported in patients with chronic HBV infection (Kramvis et al., 1997). Several studies (Lai et al., 1994 and Lee et al., 1996) they have shown stop codon (TAG) mutation (A1896) to address the emergence of HBV variants defective in HBeAg synthesis. In addition many uncommon mutations in the PreC/C region of HBV also have been described. It is known that precore variants are selected from the wild type at or after seroconversion to ani-HBe in chronic infection due to immune pressure. The selection process for HBV with mutations such as precore or core mutations can occur naturally or due to antiviral therapy. In the present study, the G to A mutation at nt 1896 was not observed in any of the HBV strains analysed. This was in contrast to the observations from our earlier study (Valliammai et al., 1995). We observed a low frequency of sequence variation in the precore region of the HBV strains in the current study.

Many studies have shown a relationship between HBV genotypes and mutations in the precore and core region (Lindh et al, 1999; Chu et al., 2002 and Lok et al., 2001). The precore stop codon mutation (G to A at nt-1896) is found associated with HBV genotypes B, C, and D but not genotype A (Chu et al., 2002 and Lok et al., 2001). We did not perform a genotypic determination for the HBV strains analysed in the present study. However, in the overall eight anti-HBe positive and HBV-DNA positive cases we have observed two of our isolates (one diagnosed having CAH with cirrhosis and another with chronic HBV infection respectively) had 'C' at nt 1858, a feature which is suggested a marker known to be present in the A or F genotypes of HBV (Li et al., 1993). In contrast, in one Indian study, the presence of "C" at nt 1858 was classified as genotype D on the basis of S gene sequence analysis (Gandhe et al., 2003). In the proximal stem of the RNA encapsidation signal or epsilon (ε), the G residue at 1896 is normally paired with a T
at 1858 in non-A genotypes but with a C in the A genotype (Kramvis et al., 1998). A G to A switch at 1896 would result in unstable base paring (A-C) at that position, destabilizing the stem-loop structure of \( \varepsilon \), and reducing the efficiency of HBV replication. In contrast, in non-A genotypes this mutation creates a Watson-Crick T(U)-A base pair, stabilizing the secondary structure of \( \varepsilon \), and enhancing viral replication. Mutation at nt-1862 (G-T, valine to phenylalanine) was seen in these two cases.

A mutation at position 1862, which occurs in the bulge of \( \varepsilon \), has been detected in asymptomatic HBV carriers, and in patients with chronic hepatitis, cirrhosis, hepatocellular carcinoma, or fulminant hepatitis (Kramvis et al., 1997 and Kramvis et al., 1998). This mutation could affect HBeAg expression at two levels.. T1862 mutation changes the signalase recognition sequence at position -3 and is proposed to abrogate the cleavage of p25 by the cellular signal peptidase thereby preventing HBe synthesis. Alternatively, this mutation might interfere with reverse transcription of pregenomic RNA. Polymerase (reverse transcriptase) acts as a primer of RNA-directed DNA synthesis by binding to the bulge of \( \varepsilon \) Although binding of the template to position 1862 is not as crucial as its binding to 1864 and 1865, the 1862 mutation may possibly decrease the efficiency of reverse transcription and hence viral replication. Encapsidation and replication of HBV may also be impaired by mutations in the upper stem and loop of \( \varepsilon \) (Kramvis et al., 1998). In our earlier study also we have reported mutation at nt-1862, but not associated with mutation at 1896. In contrast, other workers (Santantonio et al., 1991 and Li et al., 1993) observed T1862 mutation along with 1896 stop codon mutation. Even though, the presence of 'C' at nt-1858 could explain the reason, which precluded A1896 mutation in these cases, it was not clear why the remaining cases did not show A1896 mutation in their HBV precore sequences. It seems that
there could be considerable geographical variation on the occurrence of precore defective HBV variants. A mutation at 1899, which may occur in association with the 1896 mutation or other mutations that are associated with HBeAg negativity, is another mutation that improves the stability of ε by providing an additional A-T (U) base pair (Kramvis et al., 1997). In our study the precore sequence from case P1 (asymptomatic chronic carrier), and P5 (HIV/HBV coinfection) had a mutation at nucleotide position 1899 (G to A) and leading to substitution of aspartic acid for glycine at residue 29. Interestingly A1899 mutation was also seen in the HBe positive case analysed.

HBeAg is not found in some patients despite viral replication, although there is no mutation preventing the HBe Ag production on the DNA level. In these patients, additional mechanisms may exist outside the precore region. In the present study, two missense mutations at nt-1934 (T→A; Ser→Thr); at nt-1979 (A→G; Ile→Val) and at proximal core codons, 12 and 27 respectively were observed among all the HBeAg negative strains analysed while we did not observe these mutations in the HBV isolates from the HBe positive patients. Codon 12 belong to the helper T-cell epitope and codon 27 is a part of CTL epitope. Therefore, it is possible that the mutations in these codons, which belong to immune recognition sites might influence immune modulation and could contribute to development of viral persistence (Bertoletti et al., 1994). Alterations have been described in immunodominant cytotoxic T-lymphocyte epitopes corresponding to core protein in HBV chronic infections with capacity to inhibit CTL response (Carman et al., 1993). Our results are consistent with the studies from Japan (Chuwang et al., 1993) that mutations in the core region can be frequently detected in patients with chronic HBV infections and that these mutations were more often found in HBeAg negative patients. Our earlier study (Valliammai et al., 1995) and the present one suggest that
precore/core variants of HBV defective in HBeAg synthesis may be relatively common in Indian patients with chronic HBV infection. It is suggested that interferon therapy is ineffective in completely eliminating mutant HBV infection and that relapse rate is high in chronic HBV infection with precore mutant forms (Sarin et al., 1996). Therefore, the emergence of precore/core HBV mutants may have therapeutic implications in view of their potential to develop persistence and liver damage in the infected patients.

Accumulation of mutations in due course of viral persistence and due to host immune pressure could lead to the progression of liver damage. Multiple sequence variations have been observed in the core region of all the HBV strains from the HBeAg negative anti-HBe positive patients analysed in this study. Since HBV core antigen (HBcAg) is an important target for CTL attack, changes in core gene sequence may have more direct impact on the activity of HBV induced liver disease. However, factors leading to progression of severe liver damage are not understood fully. It is unlikely that the severity of illness is dependent solely on the prevailing precore/core sequence; it probably relates also to such other factors like viral load, host immune response etc. Some studies have shown an association of mutations in the preC/C region with the severity of liver disease (Ehata et al., 1992). In contrast, a recent study from India has shown that chronic or fulminant hepatitis B was not associated with precore or core HBV mutants (Gandhe et al., 2003). There appears to be particularly virulent strains, among HBV mutants, which have the activity to induce severe hepatitis such as chronic active hepatitis and fulminant hepatitis B (Kosaka et al., 1991). Such virulence might be generated by other mutations coupled with, precore region defects. Research data has suggested a direct correlation between precore stop codon mutation and mutations in the core gene (Lok et al., 2001). Even while we did not observe precore stop codon mutation,
it was notable that the two patients had less common precore mutation resulting in HBeAg negativity at nt 1862 (Kramvis et al., 1997) in the precore region and also had a high frequency of mutations (>10 mutations) in the core sequence compared to the other HBV isolates studied. Further studies to correlate the viremia level with liver disease activity in order to discriminate between anti-HBe positive patients with and without liver disease would provide more data.

Genetic alterations have been observed in the precore and core nucleotide sequences of HBV strains from the anti-HBe Indian patients analysed in the present study. In a recent Indian study, a substantial proportion of anti-HBe positive chronically infected individuals continued to circulate Pre-C-wild-type HBV (Gandhe et al., 2003) and they also observed one HBeAg positive CHB patient having a pre-C mutant, and a substantial proportion of anti-HBe positives carrying the precore wild-type strain suggests that, dominance of Pre-C mutants cannot be the sole factor responsible for the absence of HBeAg. While the most common precore stop codon mutation was not detected, there was a low frequency of precore defects in the HBV isolates analysed in this study. This indicates that core mutations can be frequently detected in patients with chronic HBV infection. Prospective studies on the sequence variations of PreC/C region of HBV genome and about the molecular mechanisms in relation to progression of liver disease would provide better understanding of the biological significance of these HBe Ag negative strains in India.

**6.6 HEPATITIS C VIRUS (HCV) GENOTYPING BY INNOLIPA HCV-II LINE PROBE ASSAY (INNOGENETICS, BELGIUM)**

Hepatitis C virus (HCV) was discovered in 1989 by the use of molecular cloning techniques through the joint efforts of the Centers for Disease Control and
Prevention (CDC) and the Chiron Corporation (Alter et al., 1999). It is estimated that 3% of the world's population has been infected with the hepatitis C virus (HCV) and 170 million chronic HCV carriers are at risk of developing liver cirrhosis and/or hepatocellular carcinoma. In India, the estimated prevalence of HCV Ab is 1.8% (WHO 1997). Choo et al. (1989) determined the first complete HCV genome sequence in 1991. Since its discovery, in 1989, HCV has become the focus of intense research for several reasons. First, HCV infection frequently becomes chronic. Secondly, there is a high burden of infection in different parts of the world. Thirdly, the absence of a suitable vaccine and the availability of antiviral therapy that is effective in only a small proportion of individuals have provoked interest in the molecular biology of this virus (Raghuraman et al., 2003). As additional genome sequences of 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). 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. On the basis of its extensive genetic heterogeneity, HCV has been divided into six major genotypes and at least 80 subtypes, and each genotype share approximately 65% sequence homology. In this system, the genotypes are numbered using Arabic numbers 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.

Genotyping schemes based on sequencing of variable genes 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.,
The line probe assay (LiPA) was developed by Innogenetics (Ghent, Belgium) to genotype HCV (Stuyver et al., 1993). The LiPA is the most common method used in clinical laboratories for HCV genotyping because it can be used with amplicons from both qualitative or quantitative Amplicor HCV tests and it is easy to perform and interpret. Mixed genotype infections are easily recognized as 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. Many genotypes 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 2a and 2c (Lau et al., 1996; Smith et al., 1995).

In our study of 16 patients without HIV association, 12 cases (75%) were of single infection. Among them, 8 (67%) corresponded to 1b followed by 4 (33%) cases of genotype 1a. The remaining 4 (25%) cases were found to be of mixed infections of which 3 (75%) cases had genotypes 3 & 4, and 1 (25%) case with genotypes 1a, 1b and 4. The breakup of the HCV genotypes in the different study groups revealed that 1b seem to be more often associated with all stages of liver diseases in this part of the country.

The global distribution pattern of HCV genotypes has shown, 1a and 1b to be prevalent in the United States (Lau et al., 1995); genotypes 1b, 2a and 2b are common in Japan; genotypes 1b and 2a in China (Okamoto et al., 1994); and genotypes 3, 1 and 4 in India (Raghuraman et al., 2003). Genotypes 1,2 and 3 are seen in Europe (Schroter et al., 2002) and Australia. (McOmish et al., 1994) Genotype 4 was seen in North Africa and the Middle East. (Ray et al., 2000) Genotype 3 was also observed in the Southeast Asian countries of Thailand,
Malaysia and Singapore; genotype 5 in South Africa; and type 6 in Hong Kong, Macau and Vietnam.

There have been few studies regarding the distribution of genotypes in Indian patients with chronic liver diseases, as well as in blood donors. In our own previous study conducted among Southern Indian individuals with diagnosis of non-A, non-B hepatitis revealed the predominance of genotype 1 (87.5%) over genotype 3 (12.5%) by the DNA sequencing method (Valliammai et al., 1995). Genotype 2b (42.5) was the most frequently encountered in Chandigarh along with genotype 3 (25%) and genotype 1 (20%) by Reverse hybridization assay (Kar et al., 2000), whereas 2b and 3 were detected in almost equal proportion in Delhi (Kar et al., 2000). Panigrahi et al., 1996 sequenced HCV strains from 11 patients with chronic liver disease and shown that 27% were genotype 1 and 64% were genotype 3 from northern India. In India, recently Das et al., 2002 reported the predominance of genotype 3 from the study on 153 HCV strains by Inno-Lipa method.

In the present study, out of 16 cases analysed, 3 cases were infected with mixed genotypes and all the three cases were associated with genotype 3 & 4. In India, the occurrence of genotype 4 which was previously previously believed to be restricted to the Middle East and Africa has been reported among 125 interferon-naïve HCV-RNA positive patients, wherein genotype 4 was detected in 6% of patients (Raghuraman et al., 2003).

In another group of three HIV/HCV coinfectcd cases analysed by us, the HCV genotype 1b was noticed in all the three cases by using Inno-Lipa HCV II line probe assay. Multiply transfused hemophiliacs, especially those coinfeccd with HIV, are another group who are prone to major changes in genotypes. This is attributed to multiple exposures to contaminated plasma concentrates, resulting in
co-existence of multiple genotypes. The dominant genotype could change as a result of selection pressures, varying rates of clearance, or the emergence of variants from extravascular sites (Eyster et al., 1999). There is some evidence that HIV infection may influence the course of HCV coinfection, accelerating the progression of chronic hepatitis C. Jarvis et al. (1994) demonstrated a greater propensity to change or subtype (or both), which may be related to loss of immune function in HCV positive hemophiliacs coinfected with HIV. Patients coinfected with the hepatitis C virus (HCV) and the human immunodeficiency virus (HIV) were studied with regard to nucleotide sequence variability in the E2/NS1 first hypervariable region of the HCV genome (Sherman et al., 1996). They have suggested that an accumulation of envelope variants in the HIV/HCV coinfected patients, which could be related to ineffective viral clearance, and may help to explain prior reports of interferon (IFN) resistance. In another cohort study on hemophilic men, Sabin et al. (1997) suggested that the association between HCV genotype and progression of HIV disease, which, if confirmed, could have important implications for the treatment and care of patients coinfected with both HIV and HCV. Samaniego et al. (1997) reported 10% of HCV genotype-1b in HIV/HCV coinfected patients and he also observed a higher score of piecemeal necrosis (OR=21.7, p=0.002) and higher stage of fibrosis (OR=17.9, p=0.004) than patients without HIV infection.

Although the HCV genotype has been associated with response to IFN therapy, (Eigen et al., 1979) the observation of different responses in patients with the same genotype and the titer of viremia suggests that other factors also may be responsible for the effectiveness of IFN therapy. Over past few years, the HCV quasispecies has been intensively investigated to determine whether the degree of genetic complexity and diversity may influence the outcome of IFN therapy. Several studies confirmed that the genetic complexity of the HCV quasispecies before
therapy correlates with the response to IFN (Koizumik et al., 1995; Kanazawa et al., 1994 and Toyoda et al., 1997). In other reports, (Hagiwara et al., 1996) however, no such association was found. First time the concept of quasispecies was introduced by Manfred Eigen et al., in 1972. He proposed this concept to describe the diverse and rapidly evolving populations of related but different RNA clones arising as a result of Darwinian evolution during the early phases of life on earth (Eigen et al., 1979 and Eigen et al., 1988).

In recent years, the gap between basic science and clinical research has been increasingly altered by the evolution of molecular medicine, a new area of investigation that has shifted the focus to the mechanisms of disease at the molecular level. The wide application of these novel techniques to human virology has dramatically advanced our knowledge of viral pathogenesis and of the complex interactions between viruses and their host. A paradigm of the collision between molecular biology and clinical medicine has been the study of the HCV quasispecies and their biologic implications, particularly for viral persistence, for drug resistance and for vaccine failure (Domingo et al., 1989 and Domingo et al., 1985). Nevertheless, this extraordinary progress has also posed new series of unresolved questions.

Recent evidences indicate that the quasispecies nature of HCV constitutes a critical strategy for the virus to survive in the host. In fact, the quasispecies represent a rapidly moving target that the host immune system is unable to fully control. Moreover, the large reservoir of genetic variants provided by the quasispecies poses a major challenge for the development of effective therapeutic and preventive measures. On the other hand, the fine molecular dissection of the HCV quasispecies has provided new hints for understanding why patients are unable to clear the virus and why some do not respond to antiviral
therapy. The complexity of HCV quasispecies as determined by the number of viral genomic species detectable in a particular individual by single stranded conformational polymorphism increases as the disease advances (Koizumik et al., 1995). Koizumik et al., (1995) have proposed that a more marked immune response leads to greater degree of hepatic injury and, by exerting immune selection, leads to increase in the number of quasispecies. They also have stated that determination of the heterogeneity of HCV at the level of quasispecies may become important to stage disease and establish prognosis even without having to perform a liver biopsy. This technique could have wider application if such technique(s) for detection of quasispecies become simplified and standardized. These insights might eventually lead to novel strategies for the control of HCV infection.

Even a decade after its discovery, HCV still continues as a complex public health problem. Despite the increasing understanding of the biologic and clinical aspects of HCV infection, this virus remains a major challenge to both virologists and physicians.