7. SUMMARY

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

♦ 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).

♦ The modes of acquiring HBV in HIV/HBV were heterosexual 78%, blood transfusion 4% and others 18% in HBV coinfection groups. The same in HIV/HCV coinfection cases were heterosexual 37%, blood transfusion 27 %, intravenous 27 % and others 9%.

♦ In the HIV/HBV coinfection patients, 87% were males and 13% were females. In the HIV/HCV coinfection, 82% of the patients were males and 18% were females. Although in our study we observed predominance of males than the females in both groups, it was not statistically significant.

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

The overall analysis has shown 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. Comparisions 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, and nutritional status. Salient details are given below:

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

- 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%).

- 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 rate of HBV replication increased with the HIV disease progression.

- In the HIV/HBV coinfected group, HBeAg positivity was 25%, 47%, and 71% in HIV disease groups A, B and C respectively, whereas anti-HBe positivity was 75%, 53%, and 29% were under HIV disease group A, B, and C respectively. Statistically significant trend between HBsAg positivity and HBV-DNA positivity was seen among the three CDC defined HIV disease groups (p=0.03).

- The HBV-DNA positivity in HBeAg positive and HBeAg negative/antiHBe positive cases of HIV/HBV coinfected patients was
86.3% and 17.3% respectively. The DNA positivity difference was highly significant (p=0.0001).

♦ In the HIV/HCV coinfected groups, the HCV-RNA positivity was found to be higher in Group-C (71%) than group B (66%) but one patient in group A also showed positivity for HCV-RNA.

♦ The randomly selected 308 HIV seropositive and HBsAg negative cases tested for HBV-DNA by PCR, none of them were found to be positive for HBV-DNA. However, 300 anti-HCV negative cases were randomly selected for HCV-RNA testing by PCR, in which 2 cases (0.6%) were positive. The RNA positivity rate in anti HCV positive cases was highly significant when compared with the seronegative cases (p=0001).

♦ Out of 40 CDC classified HIV/HBV coinfected patients, 15 patients were under ART. Among them 9 (60%) were HBeAg positive and 6 (40%) were anti-HBe positive. Out of 25 HIV/HBV coinfected patients without ART, 14 (56%) were HBeAg positive and 11 (44%) were anti-HBe positive. The overall HBV-DNA positivity was 60%, 56% in patients with ART and without ART respectively.

♦ In the 11 HIV/HCV coinfected patients 6 patients were under ART. Among them 5(60%) were anti-HCV positive and one patient was anti-HCV negative but HCV-RNA positive. Out of 5 patients without ART 4 patients were anti-HCV positive and one patient was anti-HCV negative but HCV-RNA positive. Overall 67% were HCV-RNA positive in patients under ART whereas 80% of cases without ART were HCV-RNA positive.
The overall mean of HIV-RNA load in HIV/HBV (276312.9±478580 copies/ml, Mean ± SD) was higher than that of HIV/HCV (91143.24±100147.6 copies/ml, Mean ± SD) coinfected patients whereas the HIV viral load in HIV/HCV patients was lower than in those infected with HIV alone (135247±386789 copies/ml, Mean ± SD) patients. However, the HIV-RNA viral load difference was not significant in our study groups. Similarly, the difference in the CD4, and CD8 counts in the HIV/HCV coinfected patients, HIV/HBV coinfected patients, and a patients infected with HIV only was not statistically significant. This could probably be due to the small number of cases, wide range of CD4, CD8 counts and the catch point of HIV cases being in different stages of HIV disease.

7.2.2 Liver biochemical parameters in HIV patients and the impact of hepatitis B virus (HBV) and hepatitis C virus (HCV) coinfection.

The LFT markers (transaminases) profile in the patients who were infected with HIV and in HIV/HBV coinfected patients had similar ALT and AST (ALT p=0.662 & AST p=0.290). However the analysis of 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).

Difference in ALT and AST levels was not seen in the HBV-DNA positive and negative cases of HIV/HBV and also the RNA positive and negative cases of HIV/HCV coinfected patients at any stage of the follow up. But, in the inter group analysis of both HBV-DNA positive & negative group of HIV/HBV coinfected cases when compared with HIV
alone had revealed that the coinfected group had slightly higher ALT and AST than that of only HIV infected group but the difference was not statistically significant (p= >0.07).

In the HIV/HCV coinfected group the ALT and AST were significantly higher in both RNA positive and RNA negative cases (P= < 0.05) compared to the HIV alone group. The HCV-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 hepatotoxicity than HBV coinfected group.

No discernable difference of ALT and AST levels was seen between ART & naïve groups of HIV/HBV coinfected patients but in the later stage at 24th month, statistically significant difference was seen between these two groups (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 difference of ALT and AST was seen in the group of HIV/HCV ART compared to HIV ART (statistically significant difference was seen in the 6th month only) and ART group of HIV/HBV. However, the statistical significance was seen only in the base as well as the 6th month (p<0.04). This observation clearly underscores that care should be taken on HCV coinfected patients since they seem to have persistent liver damage irrespective of their ART status.
7.2.3 The opportunistic infections in HIV patients and the impact of hepatitis B virus (HBV) and hepatitis C virus (HCV) coinfection.

In the present study we assessed the impact of coinfection with hepatotropic viruses on HIV associated opportunistic infections (OPI).

The extra pulmonary TB was significantly associated with HIV/HBV (p=0.05) and HIV/HCV (p=0.013) than in HIV alone infected cases where as the pulmonary TB (p=0.018) was seen more often with HIV alone-infected cases than coinfected patients. The most common oral manifestation, oral candidiasis was also seen more frequently in HIV/HBV (p=0.001) and HIV/HCV (p=0.001) patients than in HIV alone-infected cases.

The Pneumocystis carinii pneumonia was observed in two cases (3%) of HIV alone-infected group and only one (3%) in HIV/HBV and none in HIV/HCV coinfected group. Diarrhoea is the most common gastrointestinal symptoms in persons with HIV, especially by cryptosporidium. In our study the cryptosporidial diarrhoea was higher in HIV/HBV (7%), HIV/HCV (9%) than in HIV (3%) alone-infected cases. Among the HIV associated cutaneous manifestations, almost 19% of HIV-alone infected patients had herpeszoster followed by 9% in HIV/HCV and 7% in HIV/HBV. The herpes simplex infection was observed only in the HIV/HBV coinfected group and not in other groups.

Among the HIV associated neurologic complications, cryptococcal meningitis was higher in HIV/HBV (5%) than HIV (2%) group. Toxoplasmosis was also observed in all three groups; However the rate
was higher in HCV coinfected group (9%) followed by HBV coinfected (3%) and HIV alone infected (2%) group.

- Overall, opportunistic infections in all groups 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.

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

- In overall USG/CT scan level analysis, the abnormal liver findings were observed in 37% of HIV alone-infected patients, and the remaining 63% of them had normal liver. In the HIV/HBV coinfected cases, 45% of them have shown abnormal findings, whereas in the HIV/HCV coinfected group, 70% of them were found to have abnormal liver. It seems that liver abnormality in HCV coinfected group is higher than that of HBV coinfected group.

- However, the difference is 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 it is not statistically significant, the overall analysis reveals that HCV associated liver disease progression is seen in the HIV/HCV coinfected group compared to the HBV related liver complications in HIV/HBV coinfected group.

- In the HIV alone infected cases, 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 seen in 23% and 77% of the patients showed normal liver findings.
This observation reveals that ART plays very important role in protecting the liver compared to naïve (without ART) cases in the HIV alone infected cases.

In the HIV/HBV coinfected group, the liver abnormalities were only 40% in the naïve compared to 67% in the HIV alone-infected group. It shows that the USG a finding of liver abnormalities is low in coinfecte ART naïve group, but in patients taking ART in the coinfecte group, 54% of patients had abnormal findings. This 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, coinfecte group.

7.2.5 CD4 and CD8 counts in HIV, HBV and/or HCV coinfecte patients

Our study reveals that the overall CD4 and CD8 counts in the HIV alone-infected cases were higher than that of coinfecte 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 coinfecte patients than in HIV/HBV group concludes that the impact of HCV coinfecte was much severe than HBV coinfecte, and this may lead to faster progression of HIV associated opportunistic infectio.

The overall CD8 count in the HIV/HCV coinfecte patients was lower than HIV and in HIV/HBV coinfecte patients. The difference was not statistically significant. The overall HIV-RNA in HIV/HBV coinfecte patients was 276312.9 copies / ml, which is much higher than that of HIV alone-infected group, but the difference was not significant (p=0.22). The overall mean of HIV-RNA load in HIV/HCV coinfecte
patients was lower than HIV alone-infected patients. It shows the possibility that the coinfected patients may influence the HIV diseased condition. We tried to analyse the HBV-DNA positive and negative cases of HIV/HBV coinfected patients to see any difference in the CD4 and CD8 counts; However we could not find any difference. However no difference was seen between HCV-RNA positive and HCV-RNA negative patients also. Hence, we conclude that the viremic status of the hepatitis B and hepatitis C does not influence the CD4 and CD8 depletion.

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

♦ In the present study on chronic HBV carriers, male preponderance was observed. The Male: Female ratio was 5.5:1. Analysis of the mode of acquiring HBV in this group of asymptomatic carriers revealed play injury in 16%, hospitalization in 13%, previous h/o jaundice in 17%, surgery in 8%, unsterile needle in 19% and blood transfusion in 1%. However 26% of HBV carriers could not provide any traceable history.

♦ Out of 155 chronic HBV carrier cases, 3 (1.9%) were anti-HIV positive and all the 3 were males. In the HIV coinfected chronic HBV cases, two were HBeAg positive and also HBV-DNA positive.

♦ Out of 152 HBsAg positive cases without HIV coinfection, 124 cases were tested for HBe/antiHBe, 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.

- 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 remaining one patient had normal liver, whereas, the one HBV-DNA negative case showed the hepatomegaly with portal hyper tension (PHT).

7.4 STUDIES ON HUMAN IMMUNODEFICIENCY VIRUS (HIV) COINFECTED CHRONIC LIVER DISEASE (CLD) PATIENTS

- In the present study, 192 CLD cases were analysed of whom 45 were HP proven and 147 were USG/CT scan level confirmed chronic liver disease patients. Male & Female ratio in the 45 histopathologically proven cases was 3.5:1 and in the 147 clinically diagnosed cases it was 2.5:1.

- The overall HBV and HCV status of the 192 CLD cases revealed that 24% were HBV infected \(95\% \text{ CI 18-30}\), 13% were HCV infected \(95\% \text{ CI 8-18}\) and the remaining CLD cases were of non-B& C category \(95\% \text{ CI 56-70}\).

- The risk factors analysis of clinically diagnosed cases had revealed play injury in 8%, hospitalization in 12%, previous H/o jaundice in 17%, surgery in 2%, unsterile injection in 6%, blood transfusion in 31% and 24% of cases could not provide any traceable history. In the histopathologically proven cases risk factors analysis had revealed that
the play injury was accounted for in 13%, hospitalization in 16%, previous H/o jaundice in 11%, surgery in 13%, unsterile injections in 11%, blood transfusion in 25% and 11% of cases could not provide any traceable history.

Out of 192 CLD cases, 6 (3.1%) were anti-HIV positive, in which 3 (6.3%) were seen in HBV infected CLD cases (47). One patient (4.1%) was associated with HCV positive CLD cases (24) and the remaining 2 (1.65%) cases found in non-B, non-C CLD group. The HIV association with these three groups was not statistically significant. (HIV vs HBV p=0.701, HIV vs HCV CLD p=0.543 and HIV vs non-B non-C).

In the 3 HIV/HBV coinfected cases, 2 (67%) were HBeAg positive and 1 (33%) was anti-HBe positive. These two HBe positive cases were also positive for HBV-DNA.

The analysis of HIV coinfection in HIV/HBV positive CLD between clinically diagnosed and HP proven groups did not show any statistically significant difference (p >0.1).

The HBV DNA positivity was significantly higher in HBeAg positive CLD cases (p=0.0014). The overall HBV-DNA positivity in the HIV/HBV infected CLD cases was 67% whereas only 23% of HBV-DNA positivity was observed in HBV alone infected CLD cases.

The correlation of transaminase levels with virus association yielded equivocal results in general. However, the ALT levels were seen to be significantly higher (p=0.005 in Non-B, Non-C CLD than the other groups.
MOLECULAR STUDY ON HBV AND HCV

7.5  HEpatitis B Virus (HBV) Precore /Core Mutants Study

In the present study we examined 9 Indian HBV infected individuals presenting with different clinical manifestations for possible association with Pre-C/BCP mutants.

♦ The classical hot spot mutation of G to A at nt 1896 was not observed in any of the HBV strains analysed. We have observed that two of our isolates (one from a case of CAH with cirrhosis and another with chronic HBV infection) had 'C' at nt 1858, a feature which is suggested to be a marker known to be present in the A or F genotypes of HBV.

♦ In 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.

♦ They were mutations at multiple nucleotide positions in the core region, which were observed in the study cases. Importantly, 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.
Apart from the core missense mutations resulting in aminoacid changes, there were multiple silent mutations (more than 5 mutations) in the core region of HBV was observed in all the cases analysed in this study.

7.6 HEPATITIS C VIRUS (HCV) GENOTYPING BY INNO-LIPA HCV-II LINE PROBE ASSAY (INNOGENETICS, BELGIUM)

In the present study, 16 patients without HIV association were genotyped using Inno-Lipa HCV II line probe assay. 12 cases (75%) turned out of single HCV genotype 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 had genotypes 1a, 1b and 4.

In another group of three HIV/HCV coinfectected cases analysed by us, the HCV genotype 1b was noticed in all the three cases by using Inno-Lipa HCV II line probe assay.