7. SUMMARY

7.1 LIVER DISEASE GROUP

7.1.1 Sporadic Acute Viral Hepatitis

* 297 adult cases of acute viral hepatitis were screened for markers of hepatitis A, B, C, D and E.

* HBV alone was positive in 47.5%, HEV in 22.5%, HAV in 3%, HCV in 1.7%, HDV on HBV in 2% and HEV on HBV was seen in 3.36% of the cases. 19.8% of the cases were negative for all viral markers and grouped under non A-E.

* Higher ALT levels were recorded in acute HBV infection, HEV infection and super infection of HEV on a HBV carrier when compared with acute HCV infection (p<0.001).

7.1.2 Transfusion-associated Acute Viral Hepatitis

* 78 cases of transfusion associated-AVH, with definite history of blood transfusion, were studied.

* HBV was positive in 62.8% of the cases and HCV was found positive in 25.6% of the cases. 17.9% of the cases were categorized as Non-B,Non C.

* Significantly higher ALT levels were recorded in HBV-HCV dual infections (p<0.001).
HCV positivity due to transfusion was significantly higher when compared to sporadic AVH (p<0.001). No significant difference in the HBV positivity was observed between the sporadic AVH and transfusion associated-AVH (p>0.05).

7.1.3 Fulminant hepatic failure (FHF) and Subacute hepatic failure (SAHF)

FHF: 77 patients with fulminant hepatic failure were screened for hepatitis viral markers.

HAV and HEV were positive in 23.8% each of FHF cases. HBV was positive in 19% of the childhood FHF cases. No HCV positivity was seen in these cases.

Among the adult male FHF cases, HBV was positive in 40.7%, HEV on HBV was positive in 22.2% of the cases. HCV positivity was not observed in the present series.

Among the adult females, HEV was found positive in 31% of the cases followed by HBV in 27.6% of the cases. 1(3.4%) of the cases had HCV positivity. Even though higher mortality was generally observed in FHF cases, HEV was the leading cause of death among pregnant women with FHF (8/10=80%) followed by HBV (4/6 = 66.6%)(p<0.01). SAHF: 16 cases of SAHF were included in the study and screened for viral markers.
56.25% of the cases were positive for HBV, HDV on HBV was seen in 18.8% of the cases and HAV in 12.5% of the cases. 5 cases were negative for all viral markers.

7.1.4 Chronic liver diseases (CLD)

539 CLD cases (29-CPH; 38-CAH; and 472-Cirrhosis) were included in the study.

* Of the 539 combined cases of CPH, CAH and cirrhosis, HBV was positive in 38.5% of the cases and HCV was positive in 17.6% of the cases.

* PCR was found very useful as it had detected HBV-DNA in 97.2%, 50% and 10.45% of the HBeAg positive, HBeAg negative and HBsAg negative cases respectively.

* HCV positivity was significantly higher in the transfusion-associated CLD (40%) than the non-transfusion-associated CLD (12.8%)(p<0.001). Comparable HBV positivity was recorded in both the groups (35.5% vs 44% respectively; p>0.05).

7.1.5 Hepatocellular carcinoma (HCC)

HCC recorded higher HBV prevalence with 67% positivity and HCV positivity of 22.7%. HCV positive HCC patients were significantly older than the HBV positive cases (p<0.01).
7.2 HIGH-RISK GROUPS

7.2.1 Chronic Renal Failure (CRF) cases

165 cases with chronic renal failure undergoing haemodialysis/transfusion/transplantation were analysed for hepatitis viral markers.

* HCV was positive in 36.9% and HBV was positive in 35.1% of the cases. 37% of the cases were negative for hepatitis B and C markers. HCV positivity was higher in the Dialysis and surgery group with a history of transfusion than in the group undergoing haemodialysis alone (p<0.01).

* HCV positivity increased with the increase in transfusion units and/or number of haemodialysis (p<0.05). HBV positivity did not show any such increasing trend (p>0.05).

* Majority (77.6%) of the viral positive CRF cases had normal ALT values. 20 of the 165 cases had ongoing AVH, 10 (6%) of the cases had previous history of Jaundice. Majority (81%) of the cases never experienced an attack of jaundice.

7.2.2 Intravenous drug users (IVDU)

177 male IVDUs were included in the study.

* Majority of the IVDUs (74.5%) abused Brown sugar in an injectable form followed by tidigesic and heroin.

* HCV was the predominant infecting agent among the IVDUs with 63.8% positivity, followed by HBV (24.8%). 16.3% of the cases were Non-B, Non-C.
* ALT was normal in majority (82.4%) of the cases and only one case had ongoing AVH.

### 7.2.3 Health Care Workers

78 health care workers, comprising of Doctors, Nurses, Lab. Technicians/attenders and students were screened for hepatitis B and C markers.

* HBV positivity was recorded in 6.4% of the cases and HCV was positive in 5.1% of the cases. These figures were high when compared to the HBV (3.7%) and HCV (0.86%) positivity among blood donors.

### 7.3 BASELINE STUDY

#### 7.3.1 Voluntary blood donors

1036 voluntary blood donors were screened for HBV and HCV markers.

* HBsAg was positive in 39(3.7%) of the cases and anti-HCV positivity was seen in 9(0.86%) of the cases.

* HBV-DNA was positive in 27(69.2%) of the 39 HBsAg positive cases and HCV-RNA was present in 6(66.6%) of the 9 anti-HCV positive cases, indicative of the replicative rates of the respective viruses in healthy carriers.
7.3.2 Risk factor analysis for HCV transmission

All the 321 HCV positive cases were analysed for their risk factors.

* 37% of the cases acquired the infection due to blood transfusion (BT) in combination with haemodialysis (HD) and/or surgery (SxBT+S-19%; D+BT+S-14%; D+BT-2.8%; BT-1.24%).

* Intravenous drug abuse contributed to HCV infection in 35.2% of the cases.

* Haemodialysis and Surgery were the risk factors in 2.8% each of the HCV positive cases. Hospitalisation was the only risk factor associated with HCV infection in 8% of the cases. 0.93% of the cases were health care workers and had an occupational risk.

* 13% of the cases did not have any of the above risk factors.

7.4 DIAGNOSTIC SYSTEMS EVALUATION

7.4.1 HBV Diagnostics

200 serum samples were screened by 10 different commercially available ELISA kits to check their specificity, sensitivity, positive predictive value and negative predictive value keeping Murex HBsAg ELISA as "Gold standard".

* HBsAg detectable limit of the kits was tested using WHO controls (CDC, Atlanta, USA) and found that Lisadex was able to detect HBsAg at 0.3 ng/ml, followed by Uniform II with 0.4 ng/ml and Monolisa with 0.5 ng/ml HBsAg detectable limit. Rest of the kits detected HBsAg at concentrations of 0.6 ng/ml and above.
Only 3 kits had 100% specificity, sensitivity, positive and negative predictive value. They were Lisadex, Uniform II and Eliscaan.

Pathozyme (1.5%), Supermik (1.5%), Heprofile (1.5%), Monolisa (1.5%), Biotest(1%) and Bioelisa(0.5%) picked up false-positives. Whereas, Trans EIA, Biotest and Bioelisa missed to pick up one HBsAg positive case (0.5%).

7.4.2 HCV Diagnostics

7.4.2.1 Anti-HCV Evaluation

This study was done in 2 parts

In part I, 108 CLD cases (94-cirrhosis and 14-HCC) were tested by II Gen.ELISA (ELISA-2), III Gen.ELISA (ELISA-3), III Gen.RIBA (RIBA 3.0) and RT-PCR. In part II, 105 cases (18-AVH; 45-CRF and 42-IVDU) were analysed by ELISA-3, RIBA 3.0 and RT-PCR.

In part I analysis, the anti-HCV positivity by ELISA-2, ELISA-3 and RIBA 3.0 was found to be 4.6%, 14.8% and 16.6% respectively. RT-PCR positivity of the 108 cases was 12%. The concordance of HCV-RNA positivity with the anti-HCV positivity of ELISA-2, ELISA-3 and RIBA 3.0 was found to be 100%, 56.25% and 61% respectively.

The ELISA-2 was not found to be a useful diagnostic tool for anti-HCV as it had missed substantial number of cases and the
positivity when compared to the ELISA-3 and RIBA 3.0 was significantly low (p<0.001).

* In the second part of the study, ELISA-3 had 72.3% anti-HCV positivity, whereas the RIBA had 68.5% anti-HCV positivity and 4.7% of the cases as RIBA indeterminate. 80 (76%) of the 105 cases were HCV-RNA positive.

The concordance of HCV-RNA positivity with the anti-HCV positivity of ELISA-3 and RIBA 3.0 was found to be 86.8% and 88.8% respectively. 13 (12.3%) of the anti-HCV negative cases were found to be positive for HCV-RNA.

* As there was no significant difference in the anti-HCV positivity and HCV-RNA positivity by both the assays (p>0.05), the ELISA-3 was found on par with the RIBA 3.0.

7.4.2.2. RIBA Seroreactive pattern

272 anti-HCV reactive cases by the RIBA 3.0 were analysed in the study.

* Reactivity to all the four HCV antigens was found in 40.4% of the cases followed by reactivity to c100, c33 and c22 (27.5%) and other combinations in a lesser percentage of cases.

* HCV-RNA positivity was found to be high in anti-HCV positive cases with reactivity to c33 and/or c22.
* Analysis of individual reactivity to the 4 HCV antigens revealed 99.2% reactivity to c22, 93.75% to c33, 82.3% reactivity to c100 and only 33.8% reactivity to NS5 antigen. High reactivity to c100 and c33 suggests the genotype to be type 1.

7.4.2.3. RIBA indeterminates

58 RIBA indeterminate samples were tested for HCV-RNA and for the presence of anti-HIV antibodies and compared with 60 anti-HCV RIBA positive samples.

* HCV-RNA positivity was observed in 31 (53.4%) of the cases. c22 RIBA indeterminates had HCV-RNA positivity in 67.6% and the c33 in 57%. None of the c100 indeterminates were positive for HCV-RNA.

* The comparison of HCV-RNA positivity in anti-HIV positive and negative cases among the RIBA indeterminate cases revealed 85% HCV-RNA positivity in anti-HIV positive cases as against 36.8% HCV-RNA positivity in anti-HIV negative samples. A similar analysis among RIBA positive samples showed 100% HCV-RNA positivity in anti-HIV positive cases as against 78% in the anti-HIV negative sera.
317 HCV positive cases (positivity either by anti-HCV or HCV-RNA status) and 915 HCV negative cases were analysed for their ALT levels and tested for anti-HBc IgM antibody.

* ALT was elevated in 35.3% of the HCV positive cases and also in 27.9% of the HCV negative cases. When used as a marker of HCV infection, there was a significant difference between the positivity of ALT and anti-HCV (p<0.001). ALT was not found to be a reliable surrogate marker of HCV infection.

* anti-HBc IgM positivity was observed in 61 (19.2%) of the HCV positive cases and 214 (23.4%) of the HCV negative cases. Anti-HBc IgM also was not found to be a surrogate indicator of HCV infection as it was not positive in all or most of the HCV positive cases and the positivity was equal to that seen in HCV negative cases (p<0.001).

7.5. HCV and autoantibodies

* An in-house immunofluorescence technique for the demonstration of anti-nuclear antibodies (ANA), anti-mitochondrial antibodies (AMA) and anti-smooth muscle antibodies (ASMA) was standardised in the laboratory.
* 200 serum samples (50-HBV positive, 50-HCV positive, 50-Non-B, Non-C CLD cases and 50-blood donors) were screened for ANA, AMA and ASMA using the in-house immunofluorescence technique.

* HCV positive group had significantly higher autoantibody positivity (62%) than the HBV positive (32%), NBNC group (42%) and blood donors (2%)(p<0.001).

* HCV had significantly higher autoantibody positivity (100%) in the CPH and CAH group than the Cirrhosis group (53%)(p<0.001). Autoantibodies were significantly higher in older liver disease patients (p<0.01).

7.6. FOLLOW-UP STUDIES

**HBV** : 27 HBV positive cases (9-AVH; 4-CPH; 3-CAH; 7-Cirrhosis; 4-HCC) were followed up for a period of 6 months to 2 years and analysed for their virological status.

* HBsAg became negative in 8 of the AVH and 1 of the CPH case.

HBeAg and anti-HBe was positive in 77.7% and 18.5% of the cases respectively on entry in the study. On follow up the HBeAg and anti-HBe positivity became 28.5% and 64.2%.

* HBV-DNA was positive in 85% in the initial samples and was positive in 28.5% of the cases on follow-up.
HCV: 25 HCV positive cases (7-AVH; 2-CPH; 3-CAH; 7-Cirrhosis; 6-HCC;) were followed up for 6 months to 2 years.

* None of the AVH cases became negative for the HCV viral markers totally. All the cases had the anti-HCV reactivity throughout the study.

* Of the 7 RIBA indeterminates, 4 seroconverted to clear-cut anti-HCV positives (Mean period: 5 months).

* 2 of the CAH cases, who were on interferon, lost the c100 antibody after 24 months and the HCV-RNA became negative.

* There was no significant difference in the mortality rates of HBV and HCV positive cases (p>0.05).