SUMMARY
AND
CONCLUSIONS
The potential of cloned hepatitis B viral DNA as a reliable marker of HBV infection, was demonstrated in serum samples obtained from apparently healthy blood donors, as well as patients suffering from viral hepatitis. The merit of the assay was to put in evidence the presence of HBV DNA in serum samples that were negative for hepatitis B surface antigen, a test which is routinely performed in blood banks and hospitals to detect HBV infection.

Serum samples from apparently healthy individuals negative for surface antigen, were obtained from three different sources and analyzed for the presence of HBV, by dot blot hybridization test. Out of 202 voluntary blood donor samples obtained from Red Cross Blood Bank, New Delhi, 14 were found to be positive for HBV. Among the 150 voluntary blood donors samples obtained from G.B.Pant Hospital, New Delhi, 9 were positive. Presence of HBV was also demonstrated in healthy contacts working in Delhi Cantontment Hospital.

The sensitivity of the single step DNA hybridization assay was improved 4-6 fold with the development of a two-step sandwich hybridization procedure based on a single stranded bacteriophage M13. $5 \times 10^4$ viral particles could be detected using this improved hybridization technique. This method also explores the possibility of using M13 as an universal probe.
In order to further improve upon the sensitivity, polymerase chain reaction was employed and the presence of virus was demonstrated in samples that were negative by DNA spot test. Upto 3 viral particles could be detected using PCR.

The combined use of DNA probe test along with PCR, lead to the identification of some mutants or variants of HBV. The primer combination used for the studies spanned the region between S and core (nucleotide 630 to 2130 approx.).

Three categories of subjects were analyzed for the presence of variants of HBV. The samples chosen for the analyses were negative for all serological markers of HBV infection but positive for the presence of HBV DNA. In the voluntary blood donor category, out of 4 cases analyzed by PCR, three showed a difference (map position 630-1600) from classical HBV, as evident from the presence of an additional band smaller by about 200 bp than expected. Data obtained from cloning and sequencing of two fragments (map position 630 - 730 and 1400 - 1600) of one of the variant virus indicated the virus to be similar to ayw serotype with three point mutations in the X region.

The presence of similar alterations (an additional hybridizable fragment smaller by 200 bp) in region between S and X were detected in variants obtained from patients suffering from cirrhosis of liver. This kind of variation
is indicative of two kinds of virus populations present in the serum, one having a normal size and other having a gross deletion in the region between S and X which encompasses the enhancer sequences as well as promoter to the X gene.

On the contrary when one of the variants isolated from a patient categorized as non A - non B was analyzed for the region between S and X, only one band was detected which was smaller by about 200bp than expected from classical HBV. The region between X and core (map position 1400 - 2130) also appeared to be smaller by about 200 bp than expected. This kind of alteration indicated presence of a variant of HBV which has gross deletions in the region between S and X-core. When viewed under EM this sample revealed the presence of 42nm viral particles.

In conclusion hepatitis B viral DNA probe based hybridization, is an important, reliable and essential test for detection of HBV infection with a sensitivity of three viral particles. Using this test we have been able to pick up 5-6% of voluntary blood donors that were rendered negative by conventional serological markers and thus used for transfusion. The importance of these findings are evident as the percentage of post transfusion hepatitis is quite high in India (about 5%). These missed cases could either be of HBV or variants of HBV as this test can also detect of variants of HBV.
We have been able to identify and characterize a particular kind of variant with a deletion of 200bp in the enhancer-promoter for X region. Their existence in three category of subjects namely apparently healthy individuals, patients suffering from cirrhosis of liver and patients categorized under non A non B, suggests their possible involvement in causing disease. About 6% of the non A non B patients were found to have HBV like sequences, suggesting that a large number of NANB cases are misdiagnosed and are actually suffering from HBV related disease.

More studies are warranted in identifying the different kinds of variants of clinical significance and their immunogenic segments. This would not only lead to better diagnosis and reduce the rate of post transfusional hepatitis but also eventually lay the basis for a vaccine with wider protective capacities.