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Non-A non-B antigen and antibody detection by agar gel diffusion

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Serum samples from 60 patients of acute viral hepatitis negative for both hepatitis A and B viruses were tested for the presence of non-A non-B hepatitis viral antigens and antibody. Seventeen serum samples from small normal healthy persons were also tested under identical conditions. Significant difference was observed for transaminase values in patients positive for antibody and the antigen. Antigen was detected in acute phase sera, when SGOT-SGPT levels were significantly elevated.

Epidemic and sporadic non-A non-B hepatitis occurs to a significant extent in India[1-3]. The diagnosis of hepatitis due to this group of viruses is by exclusion of virus B and virus A infections[4]. Mori *et al*[5] demonstrated the presence of an antigen in acute phase sera, when the SGPT levels were significantly elevated, in patients of non-A non-B hepatitis. This antigen reacted with an antibody in convalescent phase sera of the patients. We have repeated this study to establish the possible identity of non-A non-B antigen-antibody system.

Material and Methods

Acute phase sera for five patients, negative for virus A and virus B by exclusion, presumed to be of non-A non-B hepatitis, were tested against convalescent sera from the same patients. The acute phase sera of 36 patients of non-A non-B hepatitis were also tested against convalescent sera from different patients. The serum samples which were strongly reactive, as judged by a clear precipitin line, were used as the reference non-A non-B antigen and antibody for subsequent screening with immunodiffusion technique.

Sixty serum samples of patients of acute viral hepatitis found negative to virus B (Ausazyme Abbott Laboratories, USA), anti-HBsAg (AUSAB, Abbott Laboratories) and negative for IgM anti-hepatitis A virus by ELISA technique[6] and 17 samples from normal healthy controls were tested for the presence of antigen-antibody system of non-A non-B hepatitis by NIH's modification of Le Bouvier's immunodiffusion method. The immunodiffusion was carried out with a gel made of 0·9 per cent (w/v) agarose

dissolved in 0·05 *M* veronal buffer *p*H 8·6. Tne test pattern used was same as described earlier[7] for HBeAg and anti-HBe. The gel plates were incubated in the humid chamber at 4°C and read daily for 4 days.

Results and Discussion

All the 17 samples from normal healthy controls were found to be non-reactive. These samples were also found to be negative for HBsAg and anti-HBsAg. Twenty-seven of the 60 samples from patients with acute viral hepatitis who were negative for HBsAg, HBsAb and IgM anti-HAV were found to be reactive for antibody against reference antigen and 12 samples were found to be reactive for the presence of antigen against reference antibody. The reaction was complete with precipitin lines of identity between the test samples and reference reagent. The test samples found positive for antigen and antibody are referred to as non-A non-B antigen and antibody respectively. The test samples (21) which were non-reactive against reference reagents are referred to be of unknown identity from hepatitis B hepatitis A and non-A non-B hepatitis.

The transaminase values and the time duration of illness were compared between the samples reactive for antibody and the antigen. In samples reactive for antibody to NANB, the mean SGOT and SGPT values were 82±90 and 150±221 Karmen Units with median value of 45 and 55 respectively. In samples reactive for antigen to NANB, the mean SGOT and SGPT values were 150±101 and 210±167 Karmen Units with median value of 120 and 200 respectively. Using a non-parametric test[8], the difference for SGOT levels between samples positive for antibody and antigen was significant at 99 per cent levels and for SGPT levels the test was significant at 95 per cent levels. Duration of jaundice in patients found positive for antibody was 8·8±7·3 wk and for antigen it was 5·4±4·5 wk, the difference being statistically not significant.

Our study supports the findings of Mori *et al*[4] regarding the presence of specific antigen-antibody system amongst patients of non-A non-B acute hepatitis. Antigen can be detected in the acute phase sera when SGOT-SGPT levels are significantly elevated. Only a small proportion (20%) in the present series showed the presence of antigen by immunodiffusion method. Antibody was detected in a higher proportion (45%) in the present series. None of the patients of non-A non-B hepatitis showed the presence of both antigen and antibody at the same time.

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