Early Diagnosis of Hepatitis B by Dane Particle Associated DNA Polymerase Assay

(Accepted 8 March 1977)

SUMMARY

We have studied prospectively 178 subjects exposed to hepatitis B and 120 haemodialysed patients for the presence of HBs antigen, e antigen and DNA polymerase as well as for anti-HBs and anti-HBe antibodies. The results suggest that the DNA polymerase assay enables us to diagnose hepatitis B earlier than the radioimmunoassay for HBs and that DNA polymerase might be present in the blood in the absence of HBs in cases of confirmed hepatitis B. A positive correlation between e antigen and DNA polymerase was observed in 83% of the patients on haemodialysis who developed hepatitis B but only in 9% of normal patients developing the same disease.

The presence of Dane particle associated DNA polymerase in the serum has been associated with hepatitis B virus replication and infectivity of the blood (Krugman et al. 1974; Alter et al. 1976). This enzymic activity has been detected late in the incubation period, before clinical disease, after exposure to hepatitis B antigen and in chimpanzees even before the appearance of HBs antigenaemia (Bradley et al. 1974).

Since Dane particle associated DNA polymerase regularly appears during the incubation period, we undertook to study the appearance of HBs antigen, e antigen and DNA polymerase in normal subjects exposed to HBs antigen to determine whether the DNA polymerase assay could be helpful in diagnosing hepatitis B earlier than could the radioimmunoassay (RIA) for HBs antigen.

From September 1974 to December 1976 we have studied prospectively 178 normal subjects who were highly exposed to HBs. These subjects included personnel of haemodialysis units and close family contacts of HBs antigen positive cases (chronic carriers of acute hepatitis B). In parallel we observed 120 patients undergoing maintenance haemodialysis and 60 control patients without history of hepatitis, blood transfusions or drug addiction. The patients were bled at approximately monthly intervals for 6 months and also 1 to 6 days after each time hepatitis was suspected. This blood sample was always collected 2 to 10 days prior to jaundice (mean time 4-6 days) and at the time e transaminase activities started to increase. All the blood samples were tested for HBs and anti-HBs by a complement fixation (CF) test and by RIA (ABBOTT AUSRIA kit); e antigen and antibody were measured by immunodiffusion using 0·4% agarose in tris buffer as described previously (Cappel et al. 1974) with 10-fold concentrated serum (Minicon S. 125 concentrator, Amicon, Holland). Standard e antigen and antiserum were kindly provided by Dr E. Nordenfeld, Lund University, Sweden. Anti-HBc antibody was measured by a CF test but only on selected cases. DNA polymerase activity was measured according to the technique described by Kaplan et al. (1973). Briefly, 1 ml samples of the serum to be tested were diluted in 4 ml of phosphate-buffered saline (PBS) and pelleted by high-speed centrifugation at 40000 rev/min in a SW 50.1 rotor for 1 h. The pellets were then resuspended in PBS and recentrifuged. The second pellets were resuspended
in 50 μl of PBS made 1% with NP 40 (Shell Co.) and 0.3% with mercaptoethanol and utilized immediately. Samples (50 μl) of the concentrated serum were then added to 200 μl of a mixture containing 0.05 μmol of dATP, dCTP and dGTP, 12 μmol of NH₄Cl, 4 μmol of MgCl₂, and 50 pmol of ³H-TTP (28 Ci/mmol) dissolved in tris buffer pH 7.5. The mixture was incubated at 35 °C and 50 μl portions were precipitated after hourly intervals for 4 h with 2 ml of 10% trichloracetic acid solution. The ³H-TTP incorporated into acid-insoluble material was counted in a scintillation spectrometer. Tests were performed in triplicate. The enzymic activity was considered specific if incubation of the test serum with anti-HBs antiserum (human origin) could precipitate and remove the DNA polymerase activity from the supernatant after low-speed centrifugation. One ml of the test serum was mixed with 1 ml of anti-HBs serum (CF titre of 256); after overnight incubation at 4 °C the mixture was centrifuged at 5000 rev/min (Martin Christ centrifuge). The supernatant was then concentrated to achieve the same concentration as in the original test and tested for DNA polymerase activity. The activity was expressed as an index representing the ratio of ³H-TTP incorporated after 3 h to that at time zero.

Table 1. Correlation between HBs antigen, e antigen and DNA polymerase among 178 normal patients highly exposed to HBs antigen, 120 haemodialysed patients and 60 controls

| Type of patient | HBs alone | HBs and e antigen | HBs and DNA polymerase | HBs + e and DNA polymerase | Total HBs+/
|-----------------|-----------|-------------------|------------------------|---------------------------|------------
| Normal          | 26        | 13                | 41                     | 8                         | 88/178     |
| Haemodialysed   | 1         | 2                 | 2                      | 15                        | 18/120     |
| Controls        | 0         | 0                 | 0                      | 0                         | 0/60       |

Table 2. Comparison between the DNA polymerase test and the RIA among 178 normal patients highly exposed to HBs antigen*

<table>
<thead>
<tr>
<th>Test for DNA polymerase</th>
<th>HBs antigen (RIA)</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>49</td>
<td>19</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>39</td>
<td>71</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>90</td>
<td>178</td>
<td></td>
</tr>
</tbody>
</table>

* The results are those obtained on serum samples collected 2 to 10 days before the jaundice (mean time 4-6 days). It shows that 19 patients negative for HBs had detectable specific hepatitis B DNA polymerase in their serum; this enzymic activity had disappeared when the patients were bled 2 or 4 weeks later.

Table 1 summarizes the results for the sera obtained 1 to 6 days after the onset of hepatitis. As shown, 88 patients of the normal group developed HBs antigen (all these patients had biochemical signs of hepatitis and 60% had clinical hepatitis). Among these patients 29% developed HBs alone, 21 (22%) developed e antigen simultaneously and 49 (55%) showed DNA polymerase activity. This high percentage of patients with DNA polymerase activity (specifically associated with Dane particles) among the HBs positive cases is not surprising since it has been demonstrated that this activity appears early in the disease or late in the incubation period and is normally transient. This latter point was also confirmed in our cases since no DNA polymerase activity could be recovered 1 month later. More intriguing was the finding among HBs negative patients of 19 cases showing Dane particle associated DNA polymerase activity (Table 2). This activity was specific for hepatitis B since it was precipitable by anti-HBs as described. Furthermore, for 18 of these 19 patients we could
prove later that they had had hepatitis B. Indeed, four of them developed HBs 2 weeks later and among the 15 remaining cases in whom no HBs could be detected 2 or 4 weeks later, anti-HBs and anti-HBc appeared in five patients and anti-HBc alone in nine additional patients. Thus, only for one of these cases were we unable to detect either HBs antigen or HBs or HBc antibodies.

Of the patients on haemodialysis 18 developed HBs antigen, all had elevated transaminase activities and three developed clinical disease. In contrast to the normal patients, almost all of them developed e antigen and DNA polymerase and seven of them became chronic carriers. None of the controls developed HBs, e antigen or DNA polymerase.

The percentage of patients with e antigen in the normal patients is much higher than that described by others (Eleftheriou et al. 1975) but this might be due to the sensitivity of the test, the higher serum concentration in our study and the time of the sampling since we were working with very early blood samples. It is probable that normally a high percentage of hepatitis B cases develop e antigenaemia since even with an insensitive method like immunodiffusion, e antibodies are reported to occur in about 14% of the patients with acute hepatitis (Nielsen, Dietrickson & Juhl, 1974).

Since it has been reported by Nielsen et al. (1974) and by Eleftheriou et al. (1975) that patients with acute hepatitis who presented e antigenaemia were at a higher risk of developing chronic hepatitis than those who did not, our patients were observed to determine the evolution of their disease. We could not confirm this observation since all our patients cleared their HBs antigen between 1 and 2 months after the onset and their liver function was normal 6 months after the onset. However, the long-term evolution of these cases is not known at present.

Our results are in agreement with other studies and confirm that DNA polymerase activity appears early in the course of the disease and is normally transient. Our data also demonstrate that specific hepatitis B associated DNA polymerase could be found in the blood in the absence of detectable HBs antigen in cases of confirmed hepatitis B infection. A similar observation has been reported by Bradley et al. (1974) in experimental infections in chimpanzees in which one of the four animals that were studied developed DNA polymerase activity in the absence of HBs antigen and produced anti-HBs later.

These findings could lead to the application of the DNA polymerase assay as a complementary test in the routine screening procedures of cases of acute hepatitis. This test should then allow an early diagnosis of hepatitis B and possibly might also detect more cases of hepatitis B.

This work was supported by a grant from the Fondation Princesse Joséphine Charlotte.

Department of Virology
Institut Pasteur du Brabant
Department of Microbiology
Hôpital Universitaire Brugmann
Department of Paediatrics
Hôpital Universitaire St. Pierre
Brussels
Belgium

R. Cappel
F. de Cuyper
D. van Beers
M. Toppet
S. Cadranel
REFERENCES


*(Received 28 January 1977)*