e Antigen-antibody system as indicator of liver damage in patients with hepatitis-B antigen

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Summary

The clinical relevance of the e antigen-antibody system was investigated in 61 people persistently positive for hepatitis-B surface antigen, including 22 healthy carriers. The e antigen was not detectable in any of the healthy carriers, whereas it was found in 15 out of 28 patients with chronic aggressive hepatitis and two out of 11 with chronic persistent hepatitis. Its presence therefore indicates chronic liver disease but its absence does not exclude it. It may prove to be a particularly useful prognostic aid in chronic persistent hepatitis, since one of the two patients in whom it was found later developed aggressive hepatitis. In contrast, e antibody is of little diagnostic help, for, though it was found mostly in healthy carriers (18; 82%), it was also detectable in 9 (23%) of the patients with chronic hepatitis.

In 13 (76%) of the patients positive for e antigen Dane particles were seen on electron microscopy, but these were also present in 5 (19%) of the patients positive for e antibody. These findings are consistent with other evidence suggesting that e antigen is not a surface component of the Dane particle, but rather an independent soluble protein manufactured by the host in response to infection with the hepatitis-B virus.

Introduction

Sera positive for hepatitis-B surface antigen (HBsAg) contain additional specificities[1 2] from which at least three subtypes of HBsAg have been recognised. The new antigen-antibody system, recently identified using Ouchterlony double immunodiffusion and designated e,[3] however, is physicochemically and serologically distinct from HBsAg and its antibody.[4] In initial studies on patients positive for HBsAg, e antigen was present in 18 out of 23 sera from patients on haemodialysis who were presumed to have chronic liver disease but in only six out of 43 patients with acute hepatitis, in which its presence is a reliable indicator of progression to chronic liver disease and persistent HBs antigenaemia.[5]

According to Nielsen *et al*,[5] the e antigen was absent in healthy carriers of HBsAg, but the histological status of these carriers was not defined. Another limitation of studies to date is the lack of information on e antibody. The present study was undertaken to assess the clinical relevance of e antigen and antibody in patients with persistent HBs antigenaemia including healthy carriers, and to define the relationship of e antigen to the presence of Dane particles in the serum.

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Patients and methods

Of the 39 patients with chronic liver disease investigated 28 had chronic aggressive hepatitis and 11 chronic persistent hepatitis, the differentiation between these two conditions being made from both the clinical criteria and appearances on liver biopsy.[6] The 22 healthy carriers of HBsAg examined had either normal histology or the minor lesion of focal parenchymal necrosis.[7]

Tests for e antigen and e antibody were performed by a standard immunodiffusion technique.[3] The two test sera used gave reactions of complete identity against reference sera kindly provided by Dr J O Nielsen, of Copenhagen. For immune electron microscopy the sera were mixed with anti-HBs precipitating antisera and incubated overnight at 4°C. After dilution with phosphate-buffered saline and centrifugation for one hour at 55 000 *g* the resulting pellets were resuspended, negatively stained with 3% phosphotungstic acid (pH 6·5), and examined on Formvar-coated grids.

Results

The e antigen was present in 17 (44%) of the patients with chronic liver disease but in none of the healthy carriers ($P<0.0001$). Furthermore, 15 (54%) of the 28 patients with chronic aggressive hepatitis had the antigen compared with only 2 (18%) of the 11 patients with chronic persistent hepatitis, a difference that just failed to reach statistical significance. In contrast e antibody was present in 18 (82%) of the 22 healthy carriers and in only 9 (23%) of the patients with chronic liver disease ($P<0.0005$).

Dane particles were detected in the aggregates from 20 of the 61 sera on immune electron microscopy. Although they were found mainly in sera positive for e antigen (13 (76%) of the 17) in proportions ranging from 2% to 13% of the total particle count, they were also found, though in smaller proportions, in 5 (19%) of the 27 sera positive for e antibody and in two of the remaining 17 sera ($P<0.005$ and $P<0.001$ respectively).

Discussion

In this study e antigen was limited to patients with chronic liver disease, and the greater frequency in chronic aggressive hepatitis than in chronic persistent hepatitis also suggests that its presence is linked with continuing liver damage. Although chronic persistent hepatitis is generally a benign, self-limiting condition with a good prognosis,[8] there are instances reported of progression to chronic aggressive hepatitis.[9] Indeed, this occurred in a patient in the present series, who, interestingly, was always positive for e antigen. Thus patients with chronic persistent hepatitis and e antigen will require particularly careful follow-up so that corticosteroids may be given at the first signs of chronic aggressive hepatitis. The presence of e antibody in the serum, however, is of less clinical relevance. In particular, it does not reliably exclude chronic liver disease.

The demonstration by Nielsen *et al*[5] of a high concentration of the 42-nm Dane particles in sera positive for e antigen but not for e antibody raised the possibility that e antigen is an

additional antigenic component of the Dane particle, which is known to have an outer coat and an inner core that are antigenically distinct.[10] The e antigen is unlikely to be related to the core, since free core antigen has not been found in the serum of patients with either acute or chronic hepatitis-B virus infection. It is also unlikely to be related to HBsAg, since it is smaller and not a lipoprotein.[4] In our study the correlation between e antigen and Dane particles was not absolute, and the latter were detected, though infrequently, in sera positive for e antibody. In some preliminary studies examination of the e antigen-antibody precipitin lines obtained by immunodiffusion with the electron microscope has failed to detect Dane particles or any other aggregates, suggesting that e is a non-particulate antigen. It therefore seems more likely that e antigen is a soluble product of hepatocytes infected with the hepatitis-B virus and is not related to the virus itself.

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References

1 Levene, C, and Blumberg, B S, *Nature*, 1969, **221**, 195.
2 Le Bouvier, G L, *Journal of Infectious Diseases*, 1971, **123**, 671.
3 Magnius, L O, and Espmarck, J A, *Journal of Immunology*, 1972, **109**, 1017.
4 Magnius, L O, *Clinical and Experimental Immunology*, 1975, **20**, 209.
5 Nielsen, J O, Dietrichson, O, and Juhl, E, *Lancet*, 1974, **2**, 913.
6 De Groote, J, *et al*, *Lancet*, 1968, **2**, 626.
7 Woolf, I L, *et al*, *Journal of Clinical Pathology*, 1974, **27**, 348.
8 Becker, M D, *et al*, *Lancet*, 1970, **1**, 53.
9 Smith, M G M, Eddleston, A L W F, and Williams, R, *Clinics in Gastroenterology*, 1975, **4**, 311.
10 Almeida, J D, Rubinstein, D, and Stott, E J, *Lancet*, 1971, **2**, 1225.

Does anaemia increase the risks to the fetus caused by smoking in pregnancy?

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Summary

A significantly greater rise in carboxyhaemoglobin concentration in response to smoking a single cigarette was shown in pregnant (3·9% increase) as opposed to non-pregnant (2·1% increase) women. This was more pronounced when anaemia was present (5·0% increase) and appeared to be inversely related to the haemoglobin concentration. We suggest that the risks to the fetus may be particularly increased when anaemia complicates pregnancy in women who smoke cigarettes.

Introduction

The hazards to the fetus from maternal cigarette smoking after the fourth month of pregnancy are well known.[1-3] The risks have been variously attributed to the different substances in cigarette smoke, although recently most attention has been centred on the carbon monoxide content, which raises the level of carboxyhaemoglobin (COHb) in the blood of smokers who inhale to as much as 16%.[4] The rise in COHb concentration in response to smoking a single cigarette is greater in women than in men,[5] this being attributed to the sex difference in haemoglobin concentration. This effect of haemoglobin may have special implications for those women who smoke in pregnancy and who are anaemic.

We have therefore compared the rise in COHb concentration after smoking a single cigarette in non-pregnant, normal pregnant, and anaemic pregnant women, particularly as anaemia is still a relatively frequent complication of pregnancy.

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Patients and methods

Three groups of women were selected for the study. All were regular cigarette smokers. The first group consisted of 10 normal pregnant women late in the second trimester of pregnancy with haemoglobin levels of over 11 g/dl. The second group consisted of 10 women also late in the second trimester but whose haemoglobin was less than 10 g/dl. Apart from anaemia at the time of admission to the study these patients were normal. In each case the anaemia was characterised by a microcytic hypochromic blood film and associated with a low serum iron and normal serum vitamin B^{12} and folate levels. The third group consisted of 10 normal non-pregnant women with normal haemoglobin levels—that is, over 11 g/dl.

The change in COHb concentration was estimated spectrophotometrically[6] in response to smoking the first cigarette of the morning, the women having rested for at least 30 minutes. A sample of venous blood was withdrawn before and two minutes after smoking the cigarette. The cigarettes were of a standard size and of a "non-mild" variety. In an effort to standardise the study the women were instructed to take a puff every 40 seconds, inhaling as deeply as possible to a total of 10 puffs.

Results

The initial COHb concentration in all instances was variable and bore no relationship to the daily cigarette consumption, and although the highest mean initial level was found in the normal, non-pregnant women, this group had the lowest mean consumption of cigarettes (see table).

In the non-pregnant group the mean rise in COHb concentration ($\pm$SE of mean) was $2{\cdot}1 \pm 0{\cdot}2\%$. A significantly greater increase was found in the normal pregnant group (mean rise $3{\cdot}9 \pm 0{\cdot}4\%$; $t = 3{\cdot}91$; $P < 0{\cdot}005$). The effect was more pronounced in the anaemic women, who had a mean rise of $5{\cdot}0 \pm 0{\cdot}2\%$ ($t = 9{\cdot}9$; $P < 0{\cdot}0005$). The difference in response between the normal pregnant and anaemic pregnant women was also significant ($t = 2{\cdot}53$; $P < 0{\cdot}5$). No correlation could be shown within each group between haemoglobin and percentage rise in COHb concentration, but when the three groups were taken as a whole a significant negative correlation was found ($r = -0{\cdot}729$; $P < 0{\cdot}001$).