Patterns of chronic liver disease in Kuwait with special reference to localisation of hepatitis B surface antigen

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SUMMARY Two hundred and fifty six consecutive liver biopsy specimens (without secondary malignancy) collected over five years were reviewed to characterise the pattern of liver diseases encountered in the Kuwait region. A relatively high proportion of chronic active hepatitis (19%) and cirrhosis (40%) was found. Localisation of HBsAg was carried out by the histochemical orcein method and the immunohistological peroxidase-antiperoxidase (PAP) procedure. The PAP technique was superior to orcein both in quality and quantity in addition to its specificity. Three immunohistological staining patterns were observed: diffuse pancytoplasmic, partial perinuclear, and peripheral cytoplasmic. The positivity rate of HBsAg in chronic hepatitis was 29% and 27%, in all cases of cirrhosis. The results of immunohistology and serology of HBsAg were compared in 52 patients in whom both tests were carried out; almost one third of chronic active liver diseases were positive by both methods. Our data clearly show the sensitivity of immunohistology and its value in detecting HBsAg, especially in retrospective studies where serology is not always available. Additionally, the data show that hepatitis B infection is often associated with the development of chronic liver disease in Kuwait.

The special histological architecture, the unique system of blood supply, and the many functions of the liver render this organ vulnerable to numerous adverse effects of diverse noxious agents—namely, microbial, endogenous, and exogenous toxins and a wide variety of drugs and other chemicals. Histopathologically, however, several aetiological factors might lead to similar changes. The best example is chronic active hepatitis, where the main histological changes may result from several diverse aetiological entities such as hepatitis B, non-A non-B infections, autoimmune disturbances, alcohol, Wilson’s disease, and certain drugs (for example, methyl dopa).1

The availability of sensitive means of serological investigation such as radioimmunoassays and enzyme linked immunoabsorbent assays has led to the elucidation of several markers in viral diseases. In addition, during the last two decades great improvements have been achieved in immunohistological methods to localise various tissue antigens, especially by the use of the various immunoenzyme procedures2 and their application on formalin fixed, paraffin embedded tissues.2-4 Such procedures enable investigators to conduct retrospective studies on stored material. This is particularly important in studies of disease aetiology in developing countries where sophisticated serological analysis has not been available. Reports from the Middle East based mainly on clinical or serological data of various liver disorders are accumulating.5-7 To our knowledge, however, there is no well documented histopathological and immunohistochemical analysis of diseases of the liver in this region. We have attempted to characterise and classify histopathologically the various liver diseases in Kuwait; to study the association of liver disease with hepatitis B viral infection by localisation of at least one marker—that is, hepatitis B surface antigen (HBsAg)—histochemically and immunohistochemically; to compare the available serological results with immunohistology; and to

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correlate the immunohistochemical staining patterns with the disease process.

Material and methods

A search of the histopathological records of the departments of pathology of the Al-Sabah Hospital and Mubarak Al-Kabeer Teaching Hospital revealed 368 liver biopsy samples showing various diseases over a period of five years (January 1978 – December 1982). One hundred and two biopsy samples with diagnoses of secondary malignancies in the liver or not enough tissue available were excluded. The remaining 256 routine histological sections as well as tissue blocks were available for special staining.

HISTOLOGICAL CHARACTERISATION

The available histological sections were routinely stained with haematoxylin and eosin, Gordon and Sweet’s rectulin, Masson’s trichrome, and periodic acid Schiff with and without diastase. Also, whenever indicated Van Geison’s and Perl’s stains were used. The histopathological diagnoses were reviewed and reclassified. Special consideration and care were taken to classify cases of chronic hepatitis and cirrhosis. The criteria used to characterise the various types of chronic hepatitis were according to those described by Scheuer with a slight modification. For classification of cirrhosis, we followed the general criteria laid down during the Fifth Pan-American Congress of Gastroenterology and those reported by the World Health Organisation Working Group on the morphology of cirrhosis.

HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL LOCALISATION OF HBsAg AND EVALUATION OF THE STAINING PATTERNS

Paraffin embedded tissues were available from 256 biopsy samples. Additional consecutive sections were cut at 5 μm thickness. Histochemical orcein staining for HBsAg was carried out according to the method of Shikata et al., while immunohistochemical localisation of the same antigen was performed by the peroxidase-antiperoxidase (PAP) technique described by Sternberger et al. but used on paraffin embedded sections as previously described.

We also attempted to describe the character of the staining of the individual cells, the distribution of positive cells in the liver lobules, and the relation of the pattern and intensity of the staining to the type of disease and its degree of activity.

SEROLOGY OF HEPATITIS B VIRUS MARKERS

Some of the biopsied patients were studied serologically for the detection of various hepatitis B virus markers—namely, hepatitis B surface antigen (HBsAg), antibody to hepatitis B surface antigen (anti-HBs), antibody to hepatitis B core (anti-HBc), hepatitis B e antigen (HBe) and antibody to hepatitis B e antigen (anti-HBe) by the virology unit of the Microbiology Department, Faculty of Medicine of Kuwait University, as part of a larger series previously reported. The results of HBsAg in sera were compared with the immunohistological results and the relation to the various types of liver diseases was also studied.

Results

HISTOPATHOLOGICAL CLASSIFICATION

Table 1 shows the distribution of the various groups of liver diseases encountered. Chronic hepatitis and cirrhosis formed the majority of the cases examined. Details of the features of primary hepatocellular carcinoma have been reported elsewhere. The miscellaneous group included heterogenous conditions—namely, the less common types of diseases in this region such as alcoholic liver disease, biliary cirrhosis, non-specific types of hepatitis where no specific aetiology could be ascertained, and a group of parasitic diseases of the liver, especially hepatic schistosomiasis, and tissues adjacent to hydatid cysts.

<table>
<thead>
<tr>
<th>Type</th>
<th>No cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolving acute hepatitis</td>
<td>6</td>
</tr>
<tr>
<td>Chronic persistent hepatitis</td>
<td>16</td>
</tr>
<tr>
<td>Chronic active hepatitis</td>
<td>55</td>
</tr>
<tr>
<td>Active cirrhosis</td>
<td>49</td>
</tr>
<tr>
<td>Established cirrhosis</td>
<td>32</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>51</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>256</td>
</tr>
</tbody>
</table>

Table 2 Histochemical and immunohistological distribution of hepatitis B surface antigen (HBsAg) in liver diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>No cases</th>
<th>No cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Orcin</td>
<td>Immunoperoxidase</td>
</tr>
<tr>
<td>Resolving acute hepatitis</td>
<td>6</td>
<td>0 (17)</td>
</tr>
<tr>
<td>Chronic persistent hepatitis</td>
<td>16</td>
<td>2 (13) (25)</td>
</tr>
<tr>
<td>Chronic active hepatitis</td>
<td>49</td>
<td>11 (23) (31)</td>
</tr>
<tr>
<td>Active cirrhosis</td>
<td>55</td>
<td>13 (24) (33)</td>
</tr>
<tr>
<td>Established cirrhosis</td>
<td>47</td>
<td>7 (15) (9)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>32</td>
<td>6 (19) (19)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>51</td>
<td>3* (6) (6)</td>
</tr>
<tr>
<td>Total</td>
<td>256</td>
<td></td>
</tr>
</tbody>
</table>

*Hepatic schistosomiasis.
HBsAg localisation by both methods in various diseases. The highest positivity rate was in active cirrhosis (33%), the positivity rate in inactive cirrhosis being 19%. In chronic hepatitis 29% were positive for HBsAg. The three positive cases in the miscellaneous group were hepatic schistosomiasis.

Three main patterns of staining of the hepatocytes bearing HBsAg were observed by the PAP method. Each pattern was more predominant in one or two types of disease, although more than one pattern was seen in few specimens. The three main staining patterns were as follows.

**Diffuse pancytoplasmic.** The entire cytoplasm was diffusely stained and the brown reaction product was usually granular. The number of positive cells varied from an individual cell randomly distributed in the lobules to a group of clustered positive hepatocytes (Fig. 1). This pattern was mainly seen in chronic persistent hepatitis and well established cirrhosis. The positive hepatocytes corresponded to the cells with "ground glass" cytoplasm seen by conventional staining methods.

**Perinuclear.** The reaction was either in the form of a crescent or a globule of positive reaction in the inner cytoplasm, usually close to the nucleus. It was usually dense and granular. The positive cells were randomly distributed in a single cell fashion (Fig. 2). This pattern was seen mainly in chronic active hepatitis with early cirrhotic changes and active cirrhosis.
Peripheral cytoplasmic. The peripheries of a few individual hepatocytes showed faint staining; usually single cells were stained with an irregular distribution in the lobules (Fig. 3). This pattern was only seen in one acute resolving hepatitis and in a few cases of the very aggressive chronic active hepatitis. It occurred in the surviving cells and was never seen in morphologically damaged ("ballooned") hepatocytes.

No nuclear staining or staining of Kupffer cells was shown. Pretreatment of sections with trypsin before the immunohistochemical staining did not increase the quality or quantity of the staining reactions.

Table 3  Immunochemistry and serology of hepatitis B surface antigen (HBsAg) in liver diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>No cases</th>
<th>No cases (%) HBsAg positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immunohistochemistry</td>
<td>Serology PAP</td>
</tr>
<tr>
<td>Chronic persistent hepatitis 6</td>
<td>1 (17)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Chronic active hepatitis and active cirrhosis 17</td>
<td>5 (29)</td>
<td>6 (35)</td>
</tr>
<tr>
<td>Established cirrhosis 14</td>
<td>3 (22)</td>
<td>5 (38)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma 5</td>
<td>1 (20)</td>
<td>3 (60)</td>
</tr>
<tr>
<td>Others 10</td>
<td>3* (33)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Total 52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Hepatic schistosomiasis  
RIA = radioimmunoassay; PAP = peroxidase-antiperoxidase

SEROLOGY VERSUS IMMUNOHISTOLOGY OF HBsAg

Table 3 shows the incidence of HBsAg positivity in 52 cases where both serology and immunohistochemistry were carried out. Almost one third of the cases of chronic active liver disease (chronic active hepatitis and active cirrhosis) were positive by the two techniques. Although serology was not available in all the cases studied immunohistologically, no positive reaction by the PAP method was encountered in any of the seronegative patients' specimens examined by both methods.

Discussion

The prevalence of hepatitis B virus antigenemia in the Middle East falls between the low prevalence areas such as Europe and the USA and the high prevalence zones such as central Africa and south east Asia. In Kuwait hepatitis B virus antigenemia was found to be 37% among patients with chronic liver disease compared with 4% in normal blood donors. The neighbouring Middle East countries the prevalence of hepatitis B virus antigenemia, though similarly high, varies from one country to another. This variability could be due to the sensitivity of the methods used for testing. Thus in Iran HBsAg was detected by agar immunodiffusion in 1-4% of rural and 2% of urban populations and in 70% of cases of acute hepatitis. In Iraq, however, HBsAg was found by haemagglutination tests in 0-66% of volunteer blood donors, in 50% of cases of chronic hepatitis, and 40% of cases of cirrhosis. In a recent study from Kuwait using radioimmunoassay and detecting all hepatitis B virus markers the prevalence was as high as 4% in volunteer blood donors, 37% in cases of chronic active liver disease and cirrhosis, 60% in cases of hepatocellular carcinoma, and 50% in cases of hepatosplenic schistosomiasis.

The reported prevalence of HBsAg in tissues also varies according to the method used and the type of material studied—such as needle biopsy, operative wedge biopsy, and necropsy specimen. In Belgium, using the immunofluorescence technique, HBsAg has been found in 55% of cases of chronic persistent hepatitis, 70% of cases of chronic aggressive hepatitis, 45% of cases of cirrhosis with little activity, and 79% of cases of cirrhosis. Ulich et al in the USA, using the PAP procedure, reported the tissue localisation of HBsAg in 77% of cases of seropositive chronic active hepatitis and in 34% of cases of chronic hepatitis in general and none in the seronegative chronic hepatitis.

In the present study we compared the less sensitive and non-specific histochemical stain—that is
In conclusion, our data are the first of their kind from the Gulf region which document the histological pattern of liver disease and demonstrate the prevalence of HBsAg in chronic liver conditions. They clearly show the value of the immunohistological localisation of HBsAg and its sensitivity in contrast to serology, especially in retrospective studies where serological results are not always available. Both serology and immunohistology may be of value in elucidating the variable progress of certain chronic liver diseases such as hepatic schistosomiasis with concomitant hepatitis B infection.22

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References


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