

## Hepatitis B core and surface antigens and delta agent in chronic liver disease in Kuwait

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**Summary.** Hepatitis B surface antigen was detected immunohistochemically in 25 out of 85 liver biopsies (29.4%) of chronic liver disease. Core antigen was also demonstrated in 9 of the 25 Hepatitis HBs Ag positive biopsies (36%). Delta agent however, was found in only one case of HBs positive chronic active hepatitis. The number of hepatocytes staining positively for HBc antigen was greater in those biopsies with the strongest staining for HBs antigen. The only case of chronic active hepatitis positive for delta agent showed that the positive staining was confined to the nuclei of few hepatocytes. The routine histology showed chronic active hepatitis with a moderate degree of inflammation. The present results confirm our previous reports that almost one third of chronic liver disease in Kuwait is associated with hepatitis B infection. Previous serological studies suggest that delta agent infection is also common; however, the present study suggest that delta agent may be a transient, and not a major, contributing factor in the progression of liver disease.

**Key words:** Hepatitis Bc and s antigens - Delta agent - Chronic liver disease

### Introduction

The pattern of chronic liver disease in Kuwait, and its association with hepatitis B infection were previously documented (Al-Nakib et al., 1982; Al Adnani and Ali, 1984). In these studies almost one third of case of chronic liver disease were positive for hepatitis B markers by serology and/or immunohistology (Al Nakib et al., 1982; Al Adnani and Ali, 1984). Recently, antibodies to delta agent (a defective RNA virus) were demonstrated in 40% of hepatitis Bs antigen positive sera in Kuwait

(Nordenfelt et al., 1983). Such results are similar to the findings in Southern Italy, where the association was first described (Rizzetto et al., 1977). It has been shown that delta agent requires the helper function of the hepatitis B virus for its replication in liver cells. Superinfection with delta agent may aggravate the underlying chronic hepatitis or change the stable state of asymptomatic carriers in such a way that these patients may develop fulminating disease (Riozzetto et al., 1983; Govindarajan et al., 1985).

Since we have shown that hepatitis B infection is commonly associated with chronic liver diseases (Al Adnani and Ali, 1984) and that there is evidence that delta agent infection is also common in this area (Nordenfelt et al., 1983) we decided to investigate its prevalence in the liver tissues of patients. We have obtained monospecific heterologous antisera to characterized human anti delta sera. With immunohistochemical techniques these antisera can be used to stain viral antigens in tissue sections. The main aim of this study was to concomitantly search for these three antigens in biopsies of chronic liver disease and to correlate their presence with the activity of the disease.

### Materials and methods

Biopsies of chronic liver disease were selected without prior knowledge of the serology results for hepatitis B virus markers. 85 paraffin-embedded blocks with enough tissue were available. 5µm thick sections were cut and used for detection of the three antigens and the appropriate controls. The indirect immunoperoxidase procedure was utilized as described elsewhere (Al Adnani, 1986), with unmasking of antigen by mild treatment by trypsin prior to incubation with the primary antibodies. Rabbit anti HBs was used at a dilution of (1/500), goat anti HBc, 1/100 (Dakopatts) and human anti-delta was diluted 1/100 (a kind gift from Dr. Govindarajan). In addition, direct immunofluorescent-labelled anti-delta antibody at a dilution of 1/50-1/100

was employed (a generous gift from Dr. Rizzetto). In each run parallel sections were incubated with normal sera, and anti HBs absorbed with purified HBs and inappropriate peroxidase conjugate. Also, known positive tissues served as positive control.

The disease activity was assessed semi-quantitatively using established criteria, i.e. by the amount of inflammatory cell infiltrate, the extent of hepatocyte degenerative changes and the associated fibrosis. This assessment was correlated with positivity for the three viral antigens. Later the case records were searched for the available serological results of hepatitis B markers.

### Results

Table 1 shows the pattern of chronic liver diseases examined with the positivity rate of the three antigens in each group. The highest HBsAg and HBcAg positivity rates were 33% and 22% respectively in active cirrhosis while delta agent was detected in 1/25 Hbs positive cases (4%); a biopsy of chronic active hepatitis. The biopsy of

this case showed moderate degree of inflammation and hepatocyte damage. HBc staining was both intranuclear and cytoplasmic in hepatocytes which also strongly stained for HBs, especially in less active diseases such as established cirrhosis with numerous ground glass cells (Fig. 1), while HBs positive cases with dense inflammatory cell infiltrate showed occasional HBc positive nuclei and no cytoplasmic staining.

The 25 immunohistological HBs positive patients were also seropositive. However, 38 sera from the remaining 60 patients were examined for HBs, all were negative. In the single delta positive biopsy, HBs containing cells were few, HBc was negative and occasional delta positive nuclei were encountered (Fig. 2).

HBs was demonstrated in 3/11 (27%) specimens of hepatic schistosomiasis but neither HBc nor delta agent were detected (Fig. 3). Control sections treated with normal serum and/or absorbed HBs positive serum or peroxidase conjugate were negative.

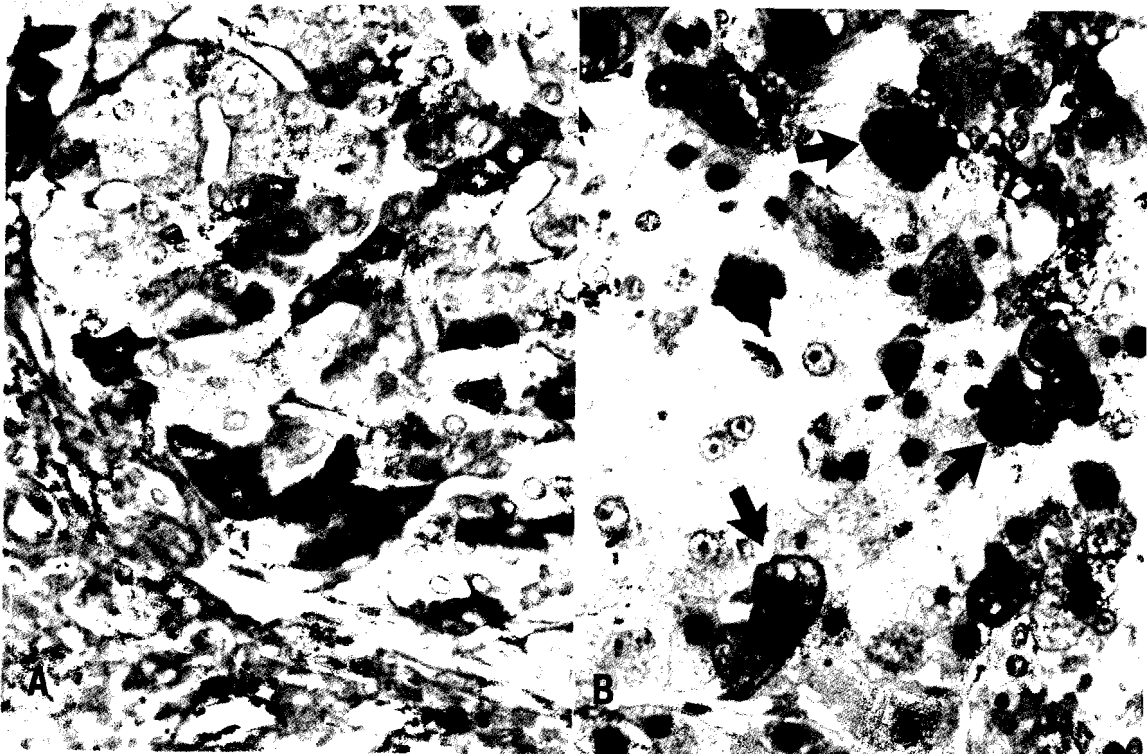
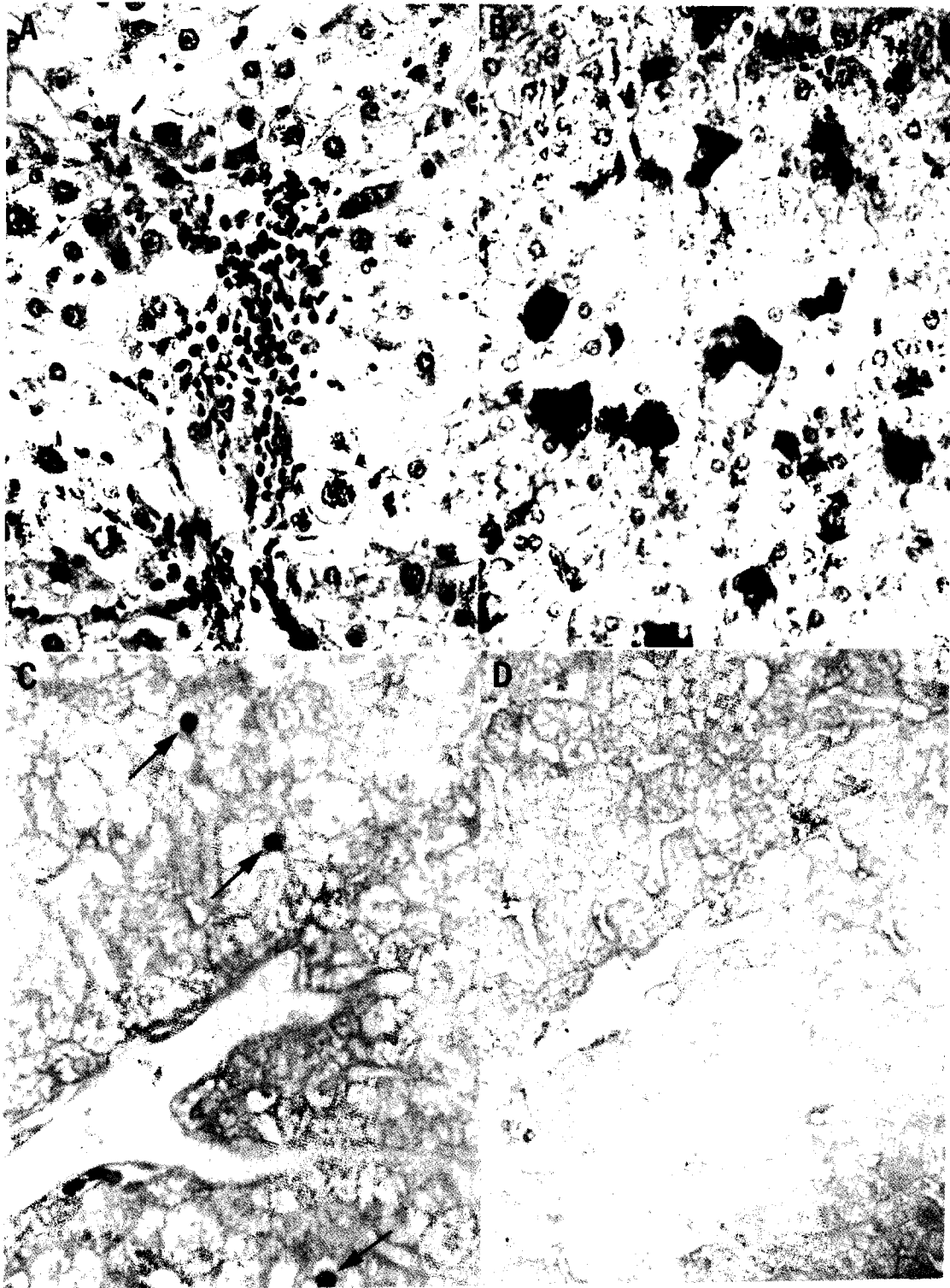
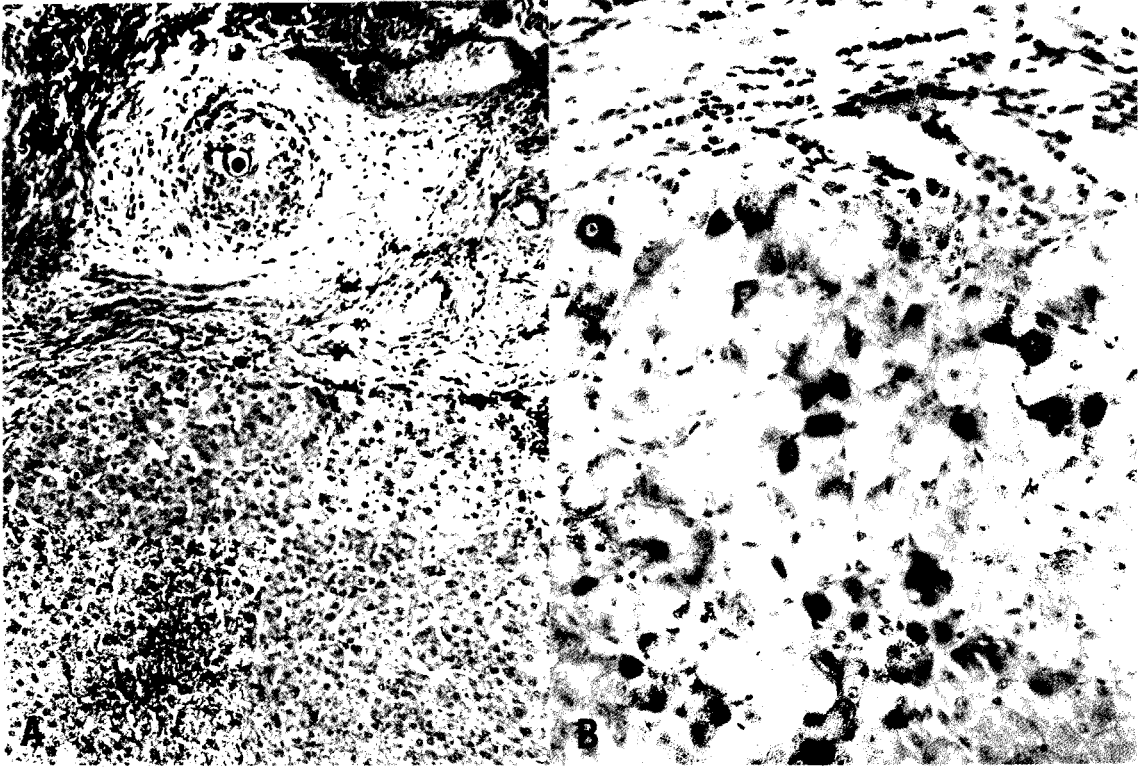


Fig. 1. Established cirrhosis. **A.** Strong positive staining for HBsAg in hepatocytes randomly distributed among negative stained cells. Immunoperoxidase - Haematoxylin  $\times 400$  **B.** Adjacent section stained for HBcAg. Note nuclear, nuclear and cytoplasmic staining (arrows) and negatively stained hepatocytes. Immunoperoxidase - Haematoxylin  $\times 400$ .



**Fig. 2.** Chronic active hepatitis. **A.** Moderate amount of portal inflammation and spilling into the parenchyma, and the hepatocytes are swollen with pale stained cytoplasm. H and E.  $\times 300$ . **B.** Scattered hepatocytes positively stained for HBsAg. Immunoperoxidase - Haematoxylin  $\times 300$ . **C.** Three positively stained nuclei for delta agent (arrows) Immunoperoxidase  $\times 300$  **D.** Parallel section to (c) stained for HbcAg. All cells are negative. Immunoperoxidase  $\times 240$



**Fig. 3.** Hepatic schistosomiasis. **A.** A wide portal zone with well formed shistosome granuloma, fibrosis and chronic inflammatory cells infiltrate. Note absence on inflammation in the hepatic parenchyma. H & E  $\times$  240. **B.** Another field from adjacent section showing positive staining for HBsAg in scattered hepatocytes. Immunoperoxidase - Hematoxylin  $\times$  240

**Table 1.** Immunohistochemical Distribution of Hepatitis B Surface and Core Antigens and Delta Agent in Chronic Liver Diseases.

Disease	No. Cases	HBs	No. and % Positive HBc	Delta
Chronic Hepatitis:				
Persistent	8	2 (25)	0	0
Active	34	10 (30)	2	1
Cirrhosis:				
Active	18	6 (33)	4 (22)	0
Established	14	4 (28)	3 (21)	0
Schistosomiasis	11	3 (27)	0	0
<b>TOTAL</b>	<b>85</b>	<b>25 (29)</b>	<b>9 (11)*</b>	<b>1 (1)**</b>

\* HBc: HBs is 9:25 (36%)

\*\*Delta: HBs is 1:25 (4%)

## Discussion

The prevalence of hepatitis Bs antigen in the normal population in Kuwait is 1.5% –4% (Al Kandari and Al-Nakib et al., 1982). In contrast HBs positivity rate in sera of chronic liver disease was found to be 37% (Al Nakib et al., 1982) and almost one third of liver biopsies were HBs positive by immunohistology (Al Adnani and Ali, 1984). This is confirmed by the present study showing that HBs Ag is positive in sera and liver biopsies of 25/85 (29%) of chronic liver disease patients. HBc Ag, however, was demonstrated in tissues for the first time in this region in 36% of the HBs sera and tissue positive patients. The previously reported positivity rate of HBc Ag in tissues differs greatly among various studies (Gudat et al., 1975; Huang, 1975; Trevisan et al., 1982). This variability may be due to a variety of causes: the population investigated, the selectivity of the cases studied, the sensitivity of the method used and nature of the material examined: for example, needle biopsy and surgical wedge biopsy studies give less material to study than the sizeable and multiple specimens obtained at autopsy. The highest positivity rate reported was 63% in biopsies of chronic active hepatitis (Ulich et al., 1985).

Our results are in agreement with the observation of Ulich et al. (1985) with regard to the number and intensity of HBc staining in hepatocytes with strong staining for HBs, and also its relation to degree of inflammation. The small number of cases studied, however, does not permit statistical analysis for significance. Similarly the results of (27%) HBsAg positive biopsies of hepatic schistosomiasis compared to 50% seropositive cases in a previous report from Kuwait (Al Nakib et al., 1985) and 42% from Egypt (Bassily et al., 1979, 1983). This association may be due to the immunosuppression especially of T-cells in patients with hepatosplenic Schistosomiasis as suggested by Ellner et al., (1980). In a study from Brazil, Lyra et al. (1976) demonstrated histological evidence of intense portal inflammation in liver biopsies of sero positive hepatic schistosomiasis in contrast to sero negative cases.

The immunohistological detection of delta agent in a single case out of 25 HBs positive (4%) is quite interesting. This is in contrast to the result of 40% serum antibody positivity in HBs positive cases of chronic active liver disease reported by Nordenfelt et al. (1983). These results suggest that delta agent in Kuwait is a common transient superimposed infection which does not appear to play a major role in development of chronic liver disease. This may be related to ethnic or genetic background as has been clearly demonstrated in various parts of the world and particularly in Italy where the disease was extensively studied (Rizzetto et al., 1977, 1979). The effect of delta infection is aggravated in intravenous drug abusers and by polytransfusion (Weller et al., 1983).

In conclusion, the present data shows that detection of HBc Ag in tissue can be a useful additional tool in the study of chronic liver disease. Delta agent, although highly prevalent, appears to be as a transient

superimposed infection in HBs positive patients, and does not seem to play a major role in the development of chronic liver disease in Kuwait.

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