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A comparative immunohistochemical study of HBsAg, HBcAg and CD57 in chronic hepatitis B pediatric patients with and without malignant disorders

Malign hastalığı olan ve olmayan çocuklarda kronik hepatit B seyrindeki farklılıkların histopatolojik ve immünohistokimyasal olarak karşılaştırılması

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Background/aims: We studied the tissue expression of hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg), as well as the presence of CD57 (+) lymphocytes in liver biopsies of 73 chronic hepatitis B pediatric patients. Twenty-seven of them had a malignant disease, either leukemia or solid tumor. We investigated the differences between the oncological and non-oncological patient groups. Methods: Liver biopsy specimens were examined for HBsAg, HBcAg and CD57 antigens immunohistochemically. Liver damage was measured according to the Knodell scoring system and all findings were assessed by Pearson correlation statistical analysis. ***Results:*** *Forty-three cases (58.9%) had minimal hepatitis, 27 cases (37%) had mild and three cases (4.1%) had moderate hepatitis. Fibrosis was shown in four cases (5.5%). For histological activity index (HAI), there was no statistical difference between the two groups. HBcAg and HBsAg expression rates were 69.9% and 90.4%, respectively. CD57 (+) lymphocytes were found in 32 cases (43.8%). HBcAg expression was higher in oncological patients. There was a positive correlation between HAI and percentage of CD57 (+) cells. However, HBsAg expression showed a negative correlation with HAI. The number of CD57 (+) cells was lower than expected.* ***Conclusion:*** *These results suggest that determination of nuclear HBcAg expression would be more useful in the follow-up of oncological patients with chronic hepatitis B.*

Key words: Chronic hepatitis B, childhood malignancy, natural killer cells

Amaç: Bu çalişmada 73 pediatrik kronik hepatit B olgusunun karaciğer biyopsilerinde histopatolojik değişiklikler, hepatit B yüzey antijeni (HBsAg), hepatit B kor antijeni (HBcAg) ve CD57 pozitif lenfosit varligi arastirilmistir. Olgularin 27'sinde ayni zamanda lösemi I lenfoma yada malign solid tümör mevcuttur. Çalismanin amaci onkolojik hastaligi olan ve olmayan gruptaki farklari irdelemektir. ***Yöntem:*** *Karaciğer biyopsilerinde HBsAg, HBcAg ve CD57 antijenleri immünohistokimyasal olarak arastirilmistir. Ayrica histopatolojik olarak incelenen biyopsilerdeki karaciğer hasari Knodell sistemine göre skorlanmis ve tüm bulgular istatistiksel olarak karsilastirilmistir.* ***Bulgular:*** *43 olguda minimal (%58.9), 27 olguda hafif (%37), 3 olguda (%4.1) orta şiddette kronik hepatit saptanmistir. Fibrozis 4 olguda (%5.5) mevcuttur. Histolojik aktivite endeksi (HAI) açisindan onkolojik hastalar ile diğer grup arasinda istatistiksel fark saptanmamistir. Sirasiyla HBcAg ve HBsAg ekspresyonu orani % 69.9 ve % 90.4 bulunmuştur. 32 olguda (%43.8) CD57 (+) lenfositler mevcuttur. Onkolojik hastalarda HBcAg ekspresyonu diğerlerinden yüksektir. Pearson analizinde HAI ile CD57 (+) lenfosit orani arasinda pozitif; HAI ile HBsAg ekspresyonu arasinda negatif korelasyon saptanmistir. Sonuç: Çalismadan elde edilen sonuçlar onkolojik hastalarda gelişen kronik hepatitlerin tani ve izleminde HbsAg yerine nükleer HBcAg'nin arastirilmasinin daha yararli olabileceğini düşündürmektedir.*

Anahtar kelimeler: Kronik Hepatitis B, Çocukluk çagi maligniteleri, natürel killer lenfositler

INTRODUCTION

Chronic hepatitis, regardless of cause, is characterized by four lesions: interface hepatitis (piecemeal necrosis), portal inflammation, lobular hepatitis and fibrosis. In the case of interface hepatitis, chronic hepatitis initially destroys the limiting plate of liver cells. In these regions, there is a predominantly mononuclear infiltration, and CD8+ suppressor/cytotoxic T-cells predominate. In contrast, the mononuclear infiltrate of portal inflammation includes predominantly CD4+ helper/inducer T-lymphocytes with an admixture of plasma cells. The lobular hepatitis term is used to determine parenchymal hepatocellular damage, which is usually much lesser in degree than that seen in acute viral hepatitis. Foci of inflammation and necrosis are located in the hepatic parenchyma,

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and the infiltrate is relatively devoid of mononuclear cells (1-4).

Immunohistochemical staining of various viral antigens such as hepatitis B core antigen (HbcAg), hepatitis B surface antigen (HbsAg) and hepatitis B e antigen (HbeAg) is widely applied. The pattern and intensity of staining in a biopsy can confirm the presence of HBV infection and can also provi de information regarding viral replication. In re cent years, clonal analysis of infiltrating lymphocytes in liver tissue in various diseases and the role of these cells have been of great interest. Especially the number and function of lymphocytes with natural killer (NK) activity have been investigated with CD 16, CD56, and CD57 markers (5-9).

It has been shown that the incidence of hepatitis B infection is higher among patients with malignant disorders than in others. Several reasons for increased risk, such as chemotherapy, exposure to ionizing radiation, blood transfusion and immune deficit have been well established. The aim of the present research was to determine HBcAg and HBsAg expressions, percentage of lymphocytes with NK marker and the extent of hepatic damage in oncology patients compared to non-oncological pediatric patients with chronic hepatitis B infection (10-11).

MATERIALS AND METHODS

Seventy-three pediatric patients representing chronic HBV infection were studied. Their liver biopsies and serum samples were analyzed. All patients had high HBV-DNA and aminotransferase levels, as well as seropositivity for HBsAg and IgG HBc antibody. The formalin fixed, paraffin embedded sections were immunohistochemically stained using monoclonal antibody CD57 (1:25 diluted, Serotec, UK), HBcAg (Serotec, UK) and HBsAg (DAKO, Denmark). Primer antibodies were visualized with a strepavidin-biotin-peroxidase kit and DAB chromogen substrate (DAKO, Denmark).

The immune staining for HBsAg and HBcAg was scored 0-2 according to intensity and quantity. On each slide, minimum of 1000 hepatocytes were observed and positive cells were counted. Three pathologists performed these assessments independently and in a blinded fashion. The number of HBsAg and HBcAg positive cells was obtained by average of the results of the three pathologists. The degree of positive cells was classified as absent, low and high according to calculated cut-point value. Cut-point values were found as 50/1000 for HBsAg and 60/1000 for HBcAg on SPSS program. Cut-point value for CD57 was not calculated because only a few cells were positive in many patients and thus it was scored as absent or present (0-1). Pearson correlation analysis was performed to determine the differences between patients with and without oncological disease.

RESULTS

The mean age was 9.67±4.11 (5-18 years) in the patients with malignant disease and 7.70±3.36 (2-16) in those without. Twenty-seven of them (37%) had undergone therapy for a malignant disease of either leukemia or solid tumor. Forty-eight cases (65.8%) were male, while 25 (34.2%) were female. According to the Knodell scoring system, 43 cases (58.9%) had minimal, 27 cases (37%) had mild and three cases (4.1%) had moderate hepatitis. Although mild fibrosis was shown in four cases (5.5%), no patient had cirrhotic changes. There was no statistical difference for grading and staging between the two patient groups.

The median of HBsAg positive cell number was 50 (0-500) and for HBcAg 80 (0-600) in the oncological patient group. In the second group, HBsAg was 40 (0-650) and HBcAg 10 (0-250). Weak staining was seen in 30 cases (41.1%) for HbsAg and in 23 cases (31.5%) for HbcAg; strong staining was seen in 36 cases (49.3%) for HBsAg and in 28 cases (38.4%) for HbcAg (Table 1). The CD57 (+) cells were seen as uniformly scattered single cells distributed throughout the hepatic parenchyma and the portal tracts. The CD57 positive cells in the portal inflammation were mainly counted. Portal CD57 positive lymphocytes were detected in only 32 cases (43.8%). In these patients, the mean number of CD57 positive cells was 15 (Figure 1).

Table 1. The mean values for age, histological activity index (HAI), HBcAg, HBsAg and CD57 positive lymphocytes in oncological patients versus others

Groups	Age	HAI	CD57(+)	HBcAg	HBsAg
Oncological patients	9.67±4.11	3.74±2.31	9±10.9	110.6±140.2	80.04±110.5
Others	7.70±3.36	4.24±2.57	20.04±40.7	30.17±50.16	80.0±130.0

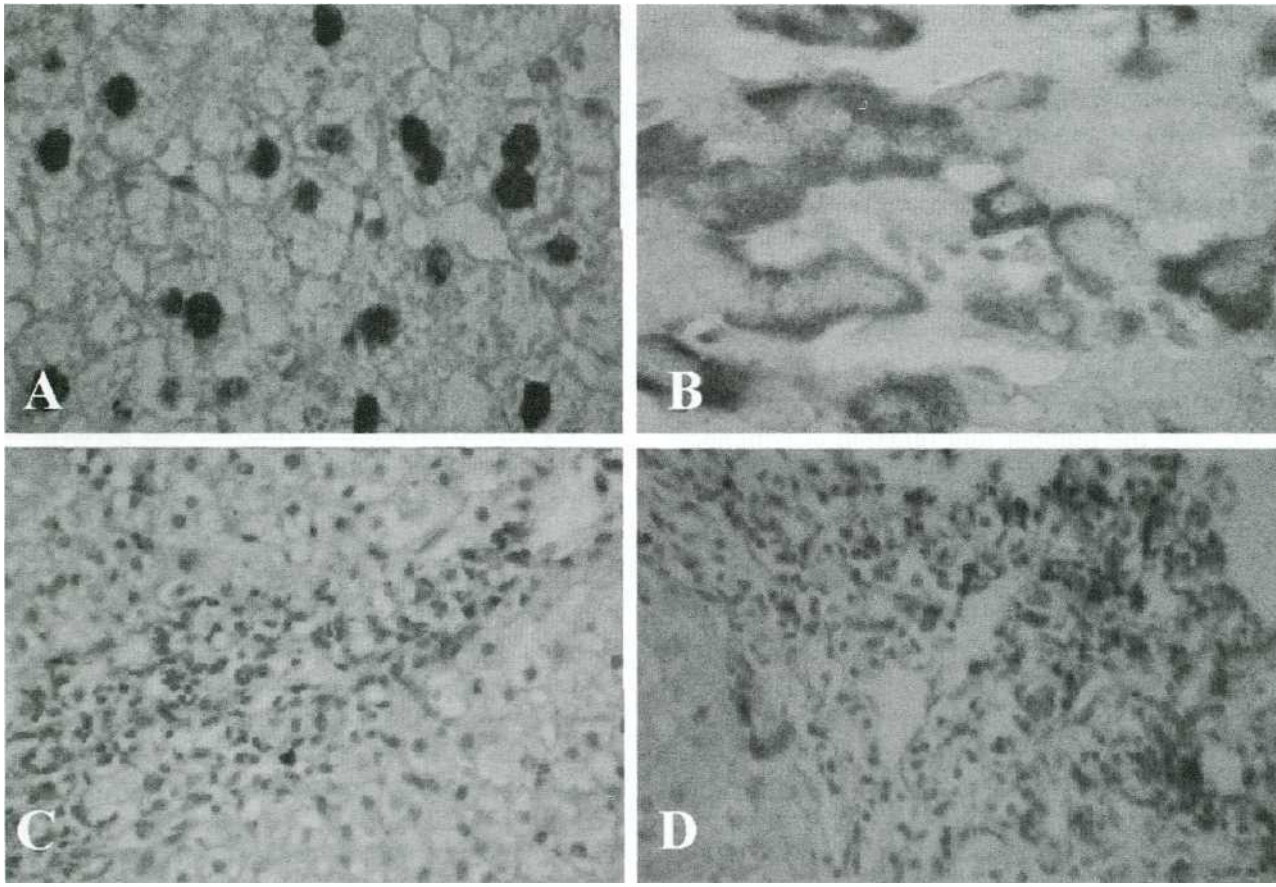

Figure 1. (a) Intensive nuclear HBcAg positivity (DAB, X400) (b) Intensive cytoplasmic HBsAg positivity (DAB, X400) (c) Portal inflammation (HE, X100) (d) CD57 positive lymphocytes in portal tract (DAB, X100)

Pearson correlation analysis test showed that the mean age was greater in oncological patients (p=0.029). There was a relationship between hepatic damage and expressions of CD57 and HBsAg. Increased histological activity index (HAI) correlated with the presence of CD57 positive cells (p=0.002) and inversely with HBsAg expression (p=0.004). There was also a positive correlation between HBsAg expression and age (p=0.033). Nuclear HBcAg expressions were higher in oncological patients group than in the other group (p=0.009) (Figures 2, 3).

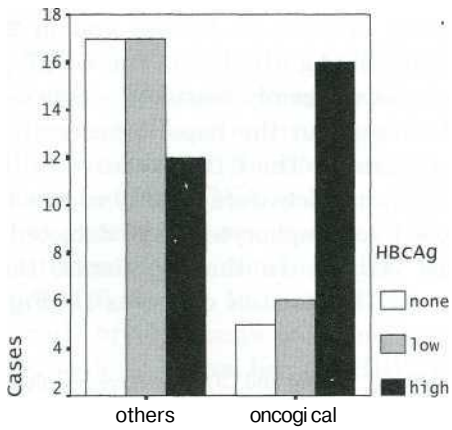

Figure 2. HBcAg status in oncological patients and others

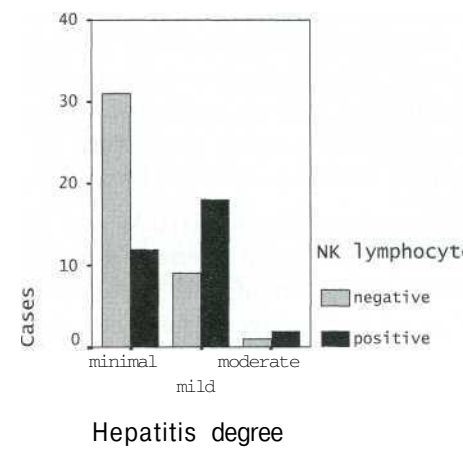

Figure 3, Natural killer cell status according to histological activity index

DISCUSSION

The generally adopted definition of chronic hepatitis B is a necroinflammatory lesion of the liver lasting for more than six months, associated with markers of hepatitis B virus (HBV) replication. In practice, the border between acute and chronic forms is not always easy to distinguish because acute viral hepatitis B is occasionally very prolonged and because some forms of chronic hepatitis develop insidiously, without an obvious acute onset. Because of increased risk of latent infection, carrier or chronic stages of hepatitis B are present in a great percentage of in-patients with malignancy (1, 2, 4, 11).

Many terms such as chronic active hepatitis (CAH), chronic persistent hepatitis (CPH) and chronic lobular hepatitis (CLH) have been used to describe chronic hepatitis in the past. It has become increasingly clear over the years that the three entities are not separate lesions but represent parts of a continuum. Several scoring systems were then developed for assessing histological activity in chronic hepatitis. The most recognized is Knodell's, which has been used extensively since its publication. The purpose of this system is determination of HAI by means of which the course of chronic hepatitis in asymptomatic patients could be followed. The HAI is based on four components: portal inflammation, periportal necrosis, lobular degeneration and fibrosis. Increasing fibrosis is now assessed as advancing stages of disease and probably results from a more active process of injury and repair. Recently it is proposed that grading (the evaluation of hepatocellular damage and inflammation) should be separated from staging (fibrosis) (12- 15).

Serological findings in chronic hepatitis of oncological patients have been widely investigated in the literature. But there are only a few comparative histopathological studies. These studies have documented that HBV infections in children with neoplastic diseases tend to take chronic forms with signs of severe inflammatory activity or hepatic stroma remodeling observed on histopathology. On the contrary, in the present study, no histopathological differences for severity of hepatitis were detected between the two patient groups (10, 11).

There was a unique clinic difference between the two patient populations, in that the mean age was greater in oncological patients. We thought that the reason for this would be associated with the age at exposure to virus. Most children without malignant disorders had acquired hepatitis B virus in-utero or in early childhood. However, oncological patients had contracted the virus during therapy of malignancy. Although a relationship was found between age and HBsAg expression, it was thought to be incidental (4, 10, 11).

Other interesting findings shown in this study were that the number of HBcAg positive patients, the intensity of HBcAg staining and the number of positive cells were higher among children with neoplastic diseases. It is well known that the presence of nuclear staining correlates well with active viral replication indicated by HBV DNA and DNA polymerase. For example, in the patients with immune deficit, diffuse staining of nuclei throughout a specimen generally suggests unbridled viral replication. In spite of this, and in agreement with previous reports, the tissue staining for HBsAg did not show significant association with HBcAg status or with HBV DNA levels, and although the viral load of HBV decreased, it showed more severe liver damage4. This suggested that viral eradication with intracellular effects of the immune response might be insufficient in patients with malignancy, and thus, that detection of HBcAg expression in the liver is more important than HBsAg. Furthermore, we found a negative correlation between HBsAg expression and hepatic damage, and the patients with higher Knodell scores showed less intensive expression of HBsAg (16-19).

Comprehensive immunological studies have suggested that resident human hepatic lymphocytes are phenotypically different from circulating lymphocytes and some of them have been shown to have the markers of natural killer (NK) lymphocytes5. These cells are defined by the expression of such adhesion molecules as CD56, CD57, and CD16. Cells with NK activity (both cytotoxic/suppressor T cells and true NK cells) are specialized subpopulations of lymphoid cells that may play an important role in the resistance to malignancy and viral infections. It has been reported that the percentage of NK cells is up to 50% of liver-associated lymphocytes. The present study indicated that the percentage of CD57 positive patients and the number of CD57 positive cells were decreased in all chronic hepatitis B patients, and that there was a positive correlation between HAI and CD57 positive cells. This result may be related to the protection mechanism for regenerating hepatocy-

tes by inhibition of hepatic NK cell activity in chronic liver diseases. This is in concordance with several investigators who have reported that genetic disorders, chronic illnesses and infections have been associated with decreased NK function injury (18-21).

In conclusion, this study indicated that detection of HBcAg expression would be more useful in diagnosis and follow-up of oncological pediatric patients with chronic hepatitis B.

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