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Liver involvement in sickle cell disease

Orak hücre hastalığında karaciğer tutulumu

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Background/aims: *Liver involvement in sickle cell disease may take place due to the primary disease itself or to secondary conditions such as iron overload, viral hepatitis and cholelithiasis. In the present study we have tried to evaluate the frequency of hepatic dysfunction and etiological factors in 48 patients with sickle cell disease.* ***Methods:*** *Clinical and laboratory investigation including liver function tests, serological tests for viral hepatitis, and abdominal ultrasonography were performed in all of the patients. Additionally, liver biopsies were taken from 13 patients.* ***Results:*** *Intrasinusoidal sickling and Kupffer cell hyperplasia were consistently seen in all of the biopsy specimens. Hepatomegaly was present in all patients, whereas liver function test abnormalities were seen in 27%. The prevalence of cholelithiasis was found as 35%. Serological tests demonstrated the presence of hepatitis B surface antigen in three, antibody to hepatitis B virus in 19 and antibody to hepatitis C virus in four of the patients. The most significant contributory finding was the presence of hemosiderosis in histological examination of liver specimens.* ***Conclusion:*** *Our data suggest that chronic liver injury in patients with sickle cell disease seems to be a multifactorial phenomenon depending mostly on overlapping factors such as iron overload and viral damage rather than primary disease itself.*

Key words: Sickle cell disease, liver involvement

Amaç: *Orak hücre hastalığında karaciğer tutulumu primer hastalık ya da aşırı demir yüklenmesi, viral hepatitler ve kolelithiasis nedeniyle gelişebilir. Çalışmamızda 48 orak hücre hastasında hepatik disfonksiyon sıklığını ve etyolojik faktörleri inceledik.* ***Yöntem:*** *Tüm hastalarda klinik bulgularla birlikte esas olarak karaciğer fonksiyon testleri olmak üzere laboratuar bulgular incelendi ve üst batın ultrasonografisi yapıldı. Ek olarak 13 hastadan karaciğer biyopsisi alındı.* ***Bulgular:*** *Biyopsi örneklerinin tümünde intrasinüzoidal oraklaşma ve Kupffer hücre hiperplazisi izlendi. Tüm hasta grubunda hepatomegali, %27 hastada ise karaciğer fonksiyon testlerinde bozukluk mevcuttu. Kolelithiasis sıklığı 35% olarak bulundu. Serolojik test sonuçlarına gore hepatit B yüzey antijeni üç, hepatit B antikor pozitifliği 19 ve hepatit C antikor pozitifliği ise toplam dört hastada tespit edildi. Karaciğer örneklerinin histolojik incelemesinde en sık izlenen bulgu hemosiderosis idi.* ***Sonuç:*** *Bulgularımız orak hücre hastalarında kronik karaciğer hasarının genellikle birden fazla nedeni olduğunu, bu faktörler arasında ise primer hastalıktan çok aşırı demir yükünün ve viral nedenlerin ön planda olduğunu düşündürmektedir.*

Anahtar kelimeler: Orak hücre hastalığı, karaciğer tutulumu

INTRODUCTION

Hepatic dysfunction is a commonly recognized complication of sickle cell disease (SCD) due to multiple factors such as intrahepatic sinusoidal sickling, bilirubin gallstones, transfusion-related hepatitis infections or excess iron deposition (1, 2). Clinical evidence of hepatic dysfunction in patients with SCD was explained by trapping of sickled cells during passage through the hepatic sinusoids which are engulfed by phagocytes causing hepatomegaly (2). Sludging and congestion of vascular beds were suggested to be the main cause of by-tissue ischemia and infarction (3). Bauer and his colleagues (4) in their retrospective study including autopsy findings of 70 SCD patients found that the spectrum of liver disease appears to be the consequence of repeated vaso-occlusive episodes. There are other studies suggesting that the main causes of liver injury in SCD patients are due to factors other than intrahepatic sickling, which was considered to be reversible, such as viral hepatitis or transfusional iron overload (5, 6).

In this study, we have investigated clinical and morphological findings of patients with SCD in order to determine the etiology and extent of hepatic abnormalities.

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MATERIALS AND METHODS

The study included 48 patients with an established diagnosis of SCD, of which six were diagnosed as S-β° thalassemia and 42 were homozygous SCD (Hb SS) patients. All the patients included were in the steady state of the disease. The diagnosis of hemoglobinopathy was established by hemoglobin electrophoresis on cellulose acetate at alkali pH; HbF was measured by the alkali denaturation method of Betke and HbA_2 by microcolumn chromatography. History of previous transfusions and alcohol or drug abuse was noted for each patient. Laboratory data including serum aminotransferases, alkaline phosphatase, total protein, albumin, bilirubin, serum ferritin, and transferrin saturation, and serological test for hepatitis B surface antigen and hepatitis B and C antibody were collected. Abdominal ultrasonography findings were noted for all of the patients. A liver biopsy was performed in eight patients with either hepatomegaly or abnormal liver enzymes, in four patients during cholecystectomy or splenectomy and in one patient postmortem. All the biopsied patients had given informed consent for the procedure. Biopsy specimens were fixed in 10% formaldehyde solution and embedded in paraffin. Histopathological sections were stained with hematoxylin-eosin, van Gieson, Gomori's trichrome, periodic acid-Schiff and Prussian blue and with Gomori's silver stain for reticulin. Additionally, the paraffin sections were stained with strep-avidin biotin technique using monoclonal mouse anti-hepatitis B surface antigen (AMO81-5M, Bio Genex). Degree of hemosiderosis was evaluated according to the deposition of hemosiderin in the Kupffer cells and hepatocytes and reported as mild, moderate or heavy. The statistical analysis was performed using Student's t and chi-square tests. Statistical significance was set at p values less than 0.05.

RESULTS

The clinical and laboratory data of the patients are summarized in Table 1. None of the patients had a history of drug or alcohol abuse. The mean (±SD) value of hematocrit was 25.7 ± 4.2% (range, 18-35%). The mean number of blood transfusions per patient was 16 units (range 0 to greater than 100 U). Mean ferritin (normal range 15-200 µg/L) was found as 837 ± 748 µg/L. Seven patients had elevated alanine aminotransferase (ALT) (normal range 0-40 IU/L) greater than 40 IU/L. The alkaline phosphatase (ALP) (normal range 5-200 IU/L) was elevated in 13 of the patients to levels above 200 IU/L. Total bilirubin was above 2 mg/dl in six of the patients. The remaining liver function tests were of no clinical significance.

Table 1. Main characteristics of the study group

	N
Gender (M/F)	29/19
Age (mean±SD)	23.7±7.3
Range	(14-43)
Sickle cell phenotype	
Hb S-β°	6
Hb SS	42
Abnormal liver function tests	
ALT ↑ (>40 IU/L)	7
Bilirubin ↑ (>2 mg/dl)	6
ALP ↑ (>200 IU/L)	13
Hepatomegaly	48
# total transfusion (U)	
None	5
< 10	15
10-50	23
> 50	5
Ferritin (µg/L)	
< 200	5
200-1000	11
> 1000	9

ALT: Alanine aminotransferase, ALP: Alkaline phosphatase

All the patients had hepatomegaly detected by both physical examination and abdominal ultrasonography. Six of the patients had been cholecystectomized before and 12 of the patients had cholelithiasis detected ultrasonographically. The prevalence of cholelithiasis was found as 35% in our study group. Serological tests demonstrated the presence of hepatitis B surface antigen in three, antibody to hepatitis B virus (HBV) in 19 and antibody to hepatitis C virus (HCV) in four of the patients.

The main characteristics of the 13 SCD patients who were biopsied are presented in Table 2. Histopathological examination of liver biopsy specimens revealed Kupffer cell hyperplasia, sickling erythrocytes, and sinusoidal dilatation in all and erythrophagocytosis in eight of them (Table 3). Mild to severe hemosiderosis was present in all but two of the biopsy materials. The two biopsies with no evidence of iron accumulation were from patients who had received no transfusion during their lifetime. All heavily hemosiderotic biopsies were of patients who had more than 40 units of transfusion history. Ferritin levels were found elevated in these patients correlated with the degree of hemosiderosis. The morphological finding of cholestasis was seen in patients with cholelithiasis.

Table 2. Clinical and laboratory data of biopsied SCD patients*

Patient	Age/Sex	RBC transfusion (U)	Ferritin (µg/L)	ALT (U/L)	Total bilirubin (mg/dl)	ALP (IU/L)	Hepatitis serology	Cholelithiasis
1	40/M	None	300	21	1.4	180	Anti Hbs/Anti HCV (+)	-
2	37/F	5	273	65	1.8	208	-	+
3	26/M	15	76	128	1.6	128	Hbs Ag(+)	+
4	22/F	5	120	38	1.8	160	-	+
5	22/F	>100	750	23	1.6	58	Anti Hbs(+)	-
6	24/F	40	>1000	21	2.1	114	Anti Hbs(+)	+
7	26/F	10	>1000	34	1.1	200	Anti Hbs(+)	-
8	17/M	None	110	20	1.8	280	-	-
9	24/M	40	937	53	1.4	124	Anti Hbs/Anti HCV (+)	-
10	23/M	15	1990	155	3.4	296	Hbs (Ag+)	+
11	14/M	NA	NA	NA	NA	NA	Hbs (Ag+)	-
12	18/M	7	3500	18	1.9	113	-	+
13	25/M	>40	700	44	1.7	137	Anti Hbs(+)	-

*ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, Anti Hbs/Anti HCV: Antibody to hepatitis B and C, HbsAg: Hepatitis surface antigen, NA: Not available

Among 48 patients, four were found to be infected with HCV. Of the 13 patients biopsied, five were serologically positive either for HBV or HCV. Two of these biopsied patients with HCV positivity had normal histological findings. Serum aminotransferase level was mildly elevated in one of these patients. One had no history of blood transfusion while the other had been moderately transfused. Of the three patients with hepatitis B, antigenemia focal necrosis and marked fibrosis were observed in biopsy specimens. Liver function tests were found abnormal in these patients. In three of the biopsy specimens, hepatitis B surface antigen was detected in the tissues. Liver function tests were abnormal and serological tests for hepatitis surface antigen were positive. Ischemic necrosis in the liver was determined in the other patient with positive HCV serology, which was the only autopsy specimen available. There was no evidence of cirrhosis in this series. Chronic active hepatitis pattern with piece-meal necrosis was seen in only one patient with positive HBV serology in whom liver function tests were also found abnormal.

DISCUSSION

In this study we examined the factors leading to hepatic dysfunction in patients with SCD. Abnormalities in liver function tests including serum

Table 3. Liver histology of biopsied SCD patients*

Patient	Liver histology	Iron	Reticulin
1	Kupffer cell hyperplasia, sinusoidal dilatation, sickling rbc, erythrophagocytosis	-	+
2	Kupffer cell hyperplasia, sinusoidal dilatation, sickling rbc, cholestasis, extramedullary hematopoiesis, mononuclear infiltration	++	+
3	Kupffer cell hyperplasia, sinusoidal dilatation, sickling rbc, cholestasis, erythrophagocytosis, hydropic degeneration of hepatocytes, portal necrosis, HbsAg(+)	+	+++
4	Kupffer cell hyperplasia, sinusoidal dilatation, sickling rbc, cholestasis	+	+
5	Kupffer cell hyperplasia, sinusoidal dilatation, sickling rbc, cholestasis, erythrophagocytosis, extramedullary hematopoiesis	+++	++
6	Kupffer cell hyperplasia, sinusoidal dilatation, sickling rbc, erythrophagocytosis, portal mononuclear infiltration	+++	+
7	Kupffer cell hyperplasia, sinusoidal dilatation, sickling rbc, erythrophagocytosis, hydropic degeneration of hepatocytes	++	+
8	Kupffer cell hyperplasia, sinusoidal dilatation, sickling rbc, hydropic degeneration of hepatocytes	-	+
9	Kupffer cell hyperplasia, sinusoidal dilatation, sickling rbc, extramedullary hematopoiesis, hydropic degeneration of hepatocytes, ischemic necrosis	++	++
10	Kupffer cell hyperplasia, sinusoidal dilatation, sickling rbc, erythrophagocytosis, cholestasis, mononuclear infiltration, chronic active hepatitis, piece-meal necrosis, HbsAg(+)	+	++
11	Kupffer cell hyperplasia, sinusoidal dilatation, sickling rbc, erythrophagocytosis, cholestasis, hydropic degeneration of hepatocytes, HbsAg(+)	+++	++
12	Kupffer cell hyperplasia, sinusoidal dilatation, sickling rbc, cholestasis	+	+
13	Kupffer cell hyperplasia, sinusoidal dilatation, sickling rbc, erythrophagocytosis	++	++

*Patients listed in the same order as in Table 2

aminotransferases, bilirubin and ALP were present in 27% of the cases. Previous studies performed in sickle cell patients confirmed that abnormal liver tests are common in these patients in the absence of a significant liver pathology (7). Hepatomegaly was a consistent finding in our study group. In the literature, presence of hepatomegaly was reported in 40-80% of living patients and in 100% of the cases in autopsy series (8).

Ultrasonographically, the pattern of liver involvement in our patients with abnormal liver function tests was similar to that seen in chronic hepatitis spectrum identified by homogeneous hyperechogenicity and speckling in the echo pattern (9, 10). Green (11) previously suggested that hepatomegaly, which is frequently seen in patients with SCD, is the result of intrasinusoidal sickling and engorgement of Kupffer cells. Our observations suggest that these morphological findings are the major contributing factors for the existence of hepatomegaly in patients with SCD; however, they do not necessarily cause hepatic dysfunction. In all of the patients with available liver specimens, sinusoidal dilatation and Kupffer cell hyperplasia were common morphological features in correlation with ultrasonography findings.

Among 48 patients, four were found to be infected with HCV. Of the 13 patients biopsied, five were serologically positive either for HBV or HCV. Recent studies have indicated that chronic HCV infection and iron overload place sickle cell patients at risk for significant liver disease and that the HCV antibody positivity is directly related to the number of transfusions given (12, 13). We could not demonstrate such an association because of the small number of biopsied patients with positive hepatitis C serology. It seems unlikely that hepatitis infections are the major risk factors for hepatic dysfunction in our series.

It is well known that patients with SCD are at increased risk for the development of cholelithiasis (4). Cholestasis was present in six of the patients on histological examination. In all, cholelithiasis was present in 35% of the patients. Similar findings were reported in other series in which prevalences ranged between 36% and 77% (7, 14, 15).

One of the most remarkable morphological findings in our series was the presence of hemosiderosis. The degree of the hemosiderosis seems to be closely related to the total amount of transfusions. All patients who were transfused with more than 40 units of blood had heavy hemosiderosis in biopsy specimens seen as iron accumulation within the parenchymal as well as Kupffer cells. Johnson and Prieto (16, 17) noted that serum ferritin levels are closely correlated with bone marrow iron stores and may also reflect hepatocellular damage. On the other hand, in another study performed by Olivieri (18), there was no correlation between the hepatic iron concentration and serum ferritin levels. The serum ferritin levels in our patients with moderate to severe hemosiderosis were found elevated to levels greater than 1000 µg/L.

Histopathological findings in our study were in accordance with previous reports stating that intrasinusoidal sickling and Kupffer cell erythrophagocytosis, seen in almost all of our patients, are nearly universal morphological features of the disease (4-6, 9). As Omata (6) also reported, there was no correlation between the degree of intrahepatic sickling and the level of liver tests in our study. Liver involvement in SCD has been studied to a great extent in autopsy series. There are a few reports with a small number of cases performed on living patients (19). Omata (6) compared liver histology from 19 living patients to autopsy specimens from 32 patients and found that presence of these morphological features was consistently seen in all specimens. There was no cirrhosis in our study group. Younger age might be an explanation since several reports previously noted that progressive liver impairment may take place in sickle cell patients with age (7).

In a recent study including 16 living children with SCD, viral damage was suggested as a probable etiology for the development of chronic hepatic lesions (20). We have observed that hemosiderosis was the most remarkable contributory factor for hepatic dysfunction. Viral causes seem to be secondarily important in our series.

Taken together, our findings and data in the literature indicate that chronic liver injury in patients with SCD seems to be a multifactorial phenomenon depending mostly on overlapping factors such as iron overload and viral damage rather than the primary disease itself. Despite recent advances in noninvasive techniques, liver biopsy remains a valuable tool for the identification of underlying etiology and correct diagnosis in SCD patients.

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