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CLINICAL STUDY

Fine Needle Aspirative Biopsy of the Liver in HBsAG-Positive Patients with End-Stage Renal Failure

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ABSTRACT

HBsAg-positive patients with end-stage renal failure have a high prevalence of asymptomatic chronic hepatitis. In order to determine the usefulness of hepatic cytology in the diagnosis of liver disease, the findings of hepatic needle core biopsy (NCB) and fine needle aspirative biopsy (FNAB) were compared in 15 HBsAg-positive uremic patients. The patients, aged 42 ± 12 years, 14 males, were on hemodialysis for periods ranging from 13 to 105 months. The NCB was processed by standard histologic and immunohistochemical techniques and FNAB by the conventional technique, using the total corrected increment score (TCI). Plasma samples were collected for evaluation of hepatic function and for viral serologic tests. In 15 patients a diagnosis was made by NCB: normal, 7 cases; chronic persistent hepatitis, 4 cases; and chronic active hepatitis, 4 cases. When the patients were allocated into two groups according to the severity of the liver histologic findings

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[group I—minor changes (normal + chronic persistent hepatitis), 11 patients; group II—major changes (chronic active hepatitis), 4 patients], statistically higher values were found in the major changes group for alanine aminotransferase (49 ± 33 vs. 24 ± 11, p = 0.04), γ-glutamyl transpeptidase [148 ± 53 vs. 38 ± 28, p < (minor) 0.02] and TCI (3.7 ± 1.2 vs. 2.5 ± 0.8, p = 0.04). In conclusion, liver FNAB can be useful as a screening procedure for the identification of liver histologic changes (minor or major) in uremic HBsAG-positive patients.

Key Words: Chronic hepatic disease; End-stage renal failure; Hemodialysis; Hepatitis B.

INTRODUCTION

Hepatitis B virus infection remains as a prevalent disease among dialysis patients in some countries (1). Probably because of their defective immune system, uremic patients frequently remain chronic antigenic carriers (2, 3). Chronic hepatitis B patients have poor prognosis after renal transplant. Progression to cirrhosis and hepatic failure are observed in recipients of long-term renal allografts (4, 5). The absence of marked increase in liver enzymes and the paucity of symptoms make difficult the diagnosis of chronic liver disease in end-stage renal failure patients (6). The current standard evaluation for suspected liver disease in these patients is liver biopsy for histology. However, the hemostatic defects of chronic renal failure may make a core biopsy a risky procedure (7).

FNAB* has been used for the diagnosis of acute allograft rejection in renal and liver transplantation (8–10). The liver FNAB allows the monitoring of *in situ* events by analysis of hepatic inflammatory infiltration as well as by morphological alterations in hepatocytes. As the hepatitis B virus is not cytopathic, the liver lesions in chronic infection are considered immune mediated (11, 12). The severity of liver lesions in chronic hepatitis B correlates with the cellular immune infiltration in the liver (12); thus the analysis of liver cytology should be useful in screening for active hepatitis B liver lesions.

In this study we compared the morphological liver data with the results of FNAB, assuming that the cytologic alterations of the parenchymal cells and the inflammatory infiltration in the liver reflect the immunological processes responsible for the pathological changes in hepatitis B. In addition, liver function tests and viral serological screening were done in the patients and correlated with the histological results.

PATIENTS AND METHODS

Patients

Seventeen chronic renal failure patients on hemodialysis, hepatitis B-positive carriers for at least 6 consecutive months and asymptomatic for liver diseases, gave informed

**Abbreviations*: FNAB, fine needle aspirative biopsy; TCI, total corrected increment; NCB, needle core biopsy; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; CPH, chronic persistent hepatitis; CAH, chronic active hepatitis.

consent for entering the study. They were in stable clinical conditions awaiting a renal transplant. A clinical evaluation and a review of the medications were done. The patients' mean age was 42 ± 12 years; 14 were male and 12 were Caucasians. The etiology of chronic renal failure was hypertension (8 patients), chronic glomerulonephritis (4), urologic obstruction (2), diabetes mellitus (1), and unknown (2). The mean period of dialysis treatment was 44 months (range 13–105) and the mean period of being HBsAg positive was 21 months (range 6–100). Two patients had a nonfunctional renal allograft and had used steroids and azathioprine for a period of 1 month and 6 years, respectively, 6 and 7 years previously. One patient had used steroids for the treatment of glomerulonephritis before beginning hemodialysis. The current medications included antihypertensive (beta-blockers, angiotensin-converting enzyme inhibitors, calcium antagonists) and oral water-soluble vitamin supplementation.

Laboratory Evaluation

Determinations of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, alkaline phosphatase (AP), γ-glutamyl transpeptidase (GGT), albumin, prothrombin time, blood platelet count, and hematocrit were done before the liver punctures. A serologic screening for hepatitis B (HBsAg, anti-HBs, HBeAg, anti-HBe, ELISA) and hepatitis C (anti-HCV, Ortho HCV 2-0 ELISA 2nd generation, recombinant c100-3, c-200, c 22-3) was also done. Hepatitis D was not tested for because there are no reported cases of it in southern Brazil. An abdominal ultrasound scan was done to exclude focal liver pathology and to guide the hepatic punctures.

Liver Punctures

The liver fine needle aspirative biopsy (FNAB) and needle core biopsy (NCB) were done sequentially in outpatients. FNAB was performed with a 25-gauge 3 1/2 needle in the 8th to 10th right intercostal space, without anesthesia, using an aspirative pistol, as described elsewhere (10). Briefly, a 20-mL syringe is attached to the pistol and to the biopsy needle, and full vacuum is applied. The material (most of it in the needle) is washed with the culture medium (RPMI 1640 + heparin 25 U/mL + Hepes 1 M 1 mL + bovine albumin 30%10 mL). Blood specimens were obtained at the same time from the fingertip for comparison with the hepatic specimens. The hepatic core biopsy was performed using a Menghini needle, in the same place as FNAB and with local anesthesia. The patients remained under close observation for a period of 6 hours. Before being discharged home, the liver ultrasound scan was repeated.

FNAB Cytology

The samples obtained from the liver FNAB and from the peripherical blood were processed for reading with a cytocentrifuge and stained with May–Grunwald–Giemsa. The integrity of hepatocyte morphology, the degree of hepatic cholestasis, and the presence and quality of the inflammatory cells in the liver and peripheral blood were evaluated by an observer blind to the patient's diagnosis. The hepatocytes were allocated in four groups according to morphological criteria: 0 = normal; 1+ = edema; 2+ = edema + vacuolation; 3+ = edema + vacuolation + intracytoplasmatic inclusions bodies; 4+ = necrosis. The cholestasis was evaluated from 0 to 4+ by deposits in the hepa-

tocytes: 0 = no cholestasis; 1+ = minimal; 2+ = light; 3+ = severe; 4+ = massive, plus "i" for intracellular and "e" for extracellular (9). The comparison between the inflammatory cells of the liver and the blood samples were used to calculate a total corrected increment (TCI), as described for transplant FNAB (8). The liver samples were estimated as representative when they contained 7 hepatocytes per 100 leukocytes, as counted in the microscope field of a contamination-free specimen. The presence of a total corrected increment ≥ 3.0 and blast cells in the FNAB cytology was considered diagnostic of immune activation using criteria similar to acute allograft rejection in liver transplants (9).

Histology

The specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin–eosin (H-E), Masson's trichrome, reticulin, PAS, and Sirius Red for analysis by light microscopy. Rabbit polyclonal anti-hepatitis B core antigen B586 and goat polyclonal anti-hepatitis B surface antigen B560 from Dako Corporation (Carpinteria, CA, USA) were used to detect the presence of viral antigens in hepatocytes. Antibody binding was visualized with an indirect immunoperoxidase technique using avidin–biotin complex.

The morphological diagnosis was done by a pathologist without prior knowledge of the patients' liver cytology data. The histological samples were classified in four groups: (*a*) normal; (*b*) chronic persistent hepatitis (CPH); (*c*) chronic active hepatitis (CAH), and (*d*) cirrhosis, as described elsewhere (13, 14). Eventually, the patients were also subdivided into two different groups according to the severity of the liver histology: group I, minor hepatic changes (normal and CPH patients); and group II, major hepatic changes (CAH patients). The needle core biopsy results were used as the standard for comparison with FNAB results.

Statistics

Data are expressed as the mean ± SD for each histologic group. Statistical analysis was performed by ANOVA, followed by Bonferroni test. A value of $p < 0.05$ was considered significant.

RESULTS

Twenty-four liver FNAB were performed in 17 patients. Two FNAB and 2 NCB were not technically adequate and were excluded from the analysis. Fifteen pairs of FNAB and NCB were available for comparative study.

Histological Results

Seven patients had normal core biopsy, 4 had chronic persistent hepatitis (CPH), and 4 chronic active hepatitis (CAH). None of the patients had cirrhosis. In all histological samples, both HBsAg and HBcAg were found in the hepatocytes by immunocytochemistry.

Table 1

Total Corrected Increment (TCI) Scores of Inflammatory Cells in Liver Obtained by FNAB in the Different Histopathologic Groups

	Normal histology	Chronic persistent hepatitis	Chronic active hepatitis
TCI	2.6 ± 0.8 (7)	2.4 ± 0.9 (4)	3.7 ± 1.2 (4)

Note. Mean ± SD; number of cases in parentheses; TCI = total corrected increment.

FNAB Results

The hepatocyte morphological score and the degree of cholestasis in the sample obtained by FNAB showed mild alterations without any differences among the three histopathologic groups. The inflammatory infiltrate of the liver was composed basically of small lymphocytes. There was mild lymphocyte infiltration in normal liver core biopsy patients, mild to moderate in chronic persistent hepatitis cases, and a moderate one in the chronic active hepatitis patients. However, blast cells and activated lymphocytes were seen only in the chronic active hepatitis patients. Large granular lymphocytes were seen in both chronic persistent hepatitis and chronic active hepatitis cytologic aspirates. The total corrected increment scores of the three different histologic groups are shown in Table 1. There was a trend for a higher mean TCI in chronic active hepatitis group compared with the other groups, but this did not reach statistical significance ($p = 0.13$).

Liver Function Tests

The serum levels of ALT and AST were high in chronic persistent and chronic active hepatitis patients (up to 2.5 times the upper normal value), but this increase was not statistically significant. The serum alkaline phosphatase was elevated in all patients, but only the serum GGT was significant higher in the chronic active hepatitis group ($p < 0.05$) (Table 2). All the other tests were consistently in the normal range for all the patients.

Serological Markers of Hepatitis Virus Infection

One patient had anti-HBs in addition to being positive for HBsAg. Nine patients (53%) were positive for HBeAg, 6 (35%) had anti-HBe, and 2 (12%) were negative for HBeAg and anti-HBe. Anti-HCV was detected in 7 (41%) patients. Six of them had histologic diagnosis of normal liver and only 1 had chronic active hepatitis.

Minor and Major Changes Groups

When the patients were subdivided based on the severity of liver histopathology, significant higher serum ALT and GGT were found in the major changes group as compared

Table 2

Liver Function Tests of the Patients in the Three Histological Groups

	Normal histology	Chronic persistent hepatitis	Chronic active hepatitis
AST (IU/L)	12 ± 4 (7)	22 ± 9 (4)	20 ± 8 (4)
ALT (IU/L)	22 ± 12 (7)	28 ± 8 (4)	49 ± 33 (4)
AP (IU/L)	151 ± 129 (7)	173 ± 126 (4)	87 ± 40 (4)
GGT (IU/L)	35 ± 24** (6)	42 ± 37* (4)	148 ± 53 (4)

Note. Mean ± SD; number of cases in parentheses. AST = aspartate transaminase; ALT = alanine transaminase; AP = alkaline phosphatase; GGT = γ-glutamyl transpeptidase.
*$p < 0.05$ or **$p < 0.01$ vs. chronic active hepatitis (ANOVA).

to the minor changes group (49 ± 33 vs. 24 ± 11, $p < 0.05$; 148 ± 53 vs. 38 ± 28, $p < 0.02$, respectively). Additionally, the total corrected increment in the major changes group was significantly higher as compared to the minor changes group (3.7 ± 1.2 vs. 2.5 ± 0.8, $p < 0.05$).

Complications

The biopsy-related complications were mild: 33% of the patients complained of transitory thoracic pain. One patient had a small subcapsular hepatic hematoma after a needle core biopsy, which was reabsorbed over the ensuing month.

DISCUSSION

The morbidity data for chronic hepatitis B virus infection in hemodialysis patients still remains inconclusive (15–17). Despite the mild biochemical alterations of liver function tests and lack of symptoms, chronic renal failure HBsAg-positive patients have shown a higher percentage of chronic liver lesions when liver histology is done (3, 18, 19). Degott et al. found chronic liver lesions in up to 84% of 51 HBsAg-positive patients on hemodialysis: 16 had chronic persistent hepatitis and 21 had chronic active hepatitis (18). Our study confirms these data in uremic hepatitis B virus "carrier" patients. We found chronic hepatitis in 54% of 15 patients, and 27% had chronic active hepatitis.

All patients, including those with normal histology, had positive results for HBsAg and HBcAg in liver histology. The presence of hepatitis B viral particles in the liver probably indicates the inability of the patients to clear the virus from the hepatocytes (20–22). Gudat et al. have shown that the presence of HBcAg in the hepatocyte is more prevalent in immune-deficient patients (23). The additional immunosuppression by drugs in renal

transplant patients can be responsible for latent virus replication and for progression of liver lesions to cirrhosis in these patients (4, 5).

The liver injury in chronic hepatitis B infection is considered immunologically mediated (11, 24). The targets for the cytotoxic lymphocytes' attack are antigenic viral particles expressed in the liver membrane cells. The antigenic stimulus in hepatitis B infection is likely to be HBcAg. It has been considered that the degree of the liver damage depends on the patient's capacity to maintain immune reaction. Partial immune dysfunction could lead to chronic persistent or chronic active hepatitis (12).

FNAB has been used to monitor liver dysfunction in hepatic transplantation, where it has shown good correlation with the core biopsy in the diagnosis of acute allograft rejection (9). The analysis of the infiltration reflects the inflammatory process in the liver. The extent of parenchymal cell lesions is measured by the morphologic and cholestasis score. The procedure is safe and reproducible, and can be done on an outpatient basis (10, 25).

To make the FNAB most useful as a method for ascertaining severity of liver disease, we divided the patients into two groups based on the severity of the liver disease on biopsy: minor and major hepatic changes. The analysis of FNAB samples in these groups has shown a significant higher total corrected increment score in major hepatic change patients ($p < 0.05$). Although the sample studied is not large enough for definitive conclusions, the FNAB technique was able to detect liver injury in these patients. However, FNAB do not replace the core biopsy, as the data from both procedures are different and complementary.

We did not observe important complications after liver FNAB. The samples were adequate in 92% of procedures, compared to 88% of the core biopsy. This was consistent with the results of others (10).

Conventional liver function tests did not help to predict the severity of the liver disease in this series or in the reports of other groups (6, 26, 27). Serum aminotransferases were mildly elevated in both chronic active and chronic persistent hepatitis patients. The high serum alkaline phosphatase found in all patients is probably a consequence of the chronic renal failure-related bone disease. Only the activity of GGT was significantly higher in chronic active hepatitis patients when compared with the other groups. Although chronic renal failure patients can have GGT elevations up to 3 times normal without hepatic disease, the chronic active hepatitis group had a fivefold increase in the GGT activity (27). This result, if confirmed by others, could be clinically useful. The large number of anti-HCV-positive patients is consistent with the high percentage of infection in hemodialysis patients described by others (28).

In conclusion, the total corrected increment score from FNAB was higher in chronic active hepatitis patients reflecting higher liver immune activation. If it is confirmed in a large series of patients, liver FNAB could be helpful as a screening procedure for detection of severe liver disease in HBsAg-positive patients on dialysis before renal transplant.

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REFERENCES

1. Mioli VA, Balestra E, Bibiano L, Carletti P, Della Bella S, Fanciulli E, Gaffi G, Marinelli R, Perilli R, Ricciatti AM, Taruscia D, Pisani E: Epidemiology of viral hepatitis in dialysis centers: A national survey. *Nephron* 61:272–283, 1992.
2. Ribot S, Rothstein M, Goldblat M, Grasso M: Duration of hepatitis B surface antigenemia (HBsAg) in hemodialysis patients. *Arch Intern Med* 139:178–180, 1979.
3. Lofgren B, Nordenfelt E, Lindholm T, Lindergard B: A ten-year follow-up of a hepatitis B epidemic in a dialysis unit. *Scand J Infect Dis* 14:165–169, 1982.
4. Parfrey PS, Farge D, Clarke Forbes RD, Dandavino R, Kenick S, Guttmann RD: Chronic hepatitis in end-stage renal disease: Comparison of HBsAg-negative and HBsAg-positive patients. *Kidney Int* 28:959–967, 1985.
5. Rao KV, Anderson WR: Liver disease after renal transplantation. *Am J Kidney Dis* 19:496–501, 1992.
6. Crosnier J, Degos F, et al: Dialysis associated hepatitis. In *Replacement of Renal Function by Dialysis* (Maher JF, ed), 3rd ed. Dordrecht: Kluwer, 1989, pp. 881–903.
7. Jubelirer SJ: Hemostatic abnormalities in renal disease. *Am J Kidney Dis* 5:219–225, 1985.
8. Hayry P, von Willebrand E: Practical guidelines for fine needle aspiration biopsy of human renal allografs. *Ann Clin Res* 13:288–306, 1981.
9. Lautenschlager I: Fine needle aspiration biopsy in liver transplants. *Transplatn Proc* 21:3618–3620, 1989.
10. Hockerstedt K, Lautenschlager I: Fine needle aspiration biopsy in liver transplants. *Transplant Proc* 21:3625–3626, 1989.
11. Thomas HC: Immunologic aspects of liver disease. In *Disease of the Liver* (Schiff L, Schiff ER, eds), 6th ed. Philadelphia: JB Lippincott, 1987, pp. 163–185.
12. Peters M, Vierling J, Gershwin ME, Milich D, Chisari FV, Hoofnagle JH: Immunology and the liver. *Hepatology* 13:977, 1991.
13. Scheuer PJ: *Liver Biopsy Interpretation*, 4th ed. London: Balliere Tindall, 1988, pp 113–130.
14. Hoofnagle J, Goodman Z: Liver biopsy—Interpretation for the 1990's. In *American Association for the Study of Liver Diseases*, Postgraduate Course, Chicago, 1991.
15. Harnett JD, Parfrey PS, Kennedy M, Zeldis JB, Steinman TI, Guttmann RD: The long-term outcome of hepatitis B infection in hemodialysis patients. *Am J Kid Dis* 11:210–213, 1988.
16. Josselson J, Kyser BA, Matthew RW, Sadler JH: Hepatitis B surface antigenemia in a chronic hemodialysis program: Lack of influence on morbidity and mortality. *Am J Kidney Dis* 9:456–561, 1987.
17. Shusterman N, Singer I: Infection hepatitis in dialysis patients. *Am J Kidney Dis* 9:447–455, 1987.
18. Degott C, Degos F, Jungers P, Naret C, Courouce AM, Potet F, Crosnier J: Relationship between liver histopathological changes and HBsAg in 111 patients treated by long-term hemodialysis. *Liver* 3:377–384, 1983.
19. Simon P, Herry D, Brissot P, Cougard A, Meyrier A: Long-term follow-up of chronic hepatitis by serial liver biopsies in HBs positive haemodialysis patients. Role of hepatotoxic drugs. *Proc Eur Dial Transplant Assoc* 15:596–598, 1978.
20. Lee BW, Yap HK, Tan M, Guan R, Quak SH, Choong L, Murugasu B, Woo KT, Jordan SC: Cell-mediated immunity in patients on hemodialysis: Relationship with hepatitis B carrier status. *Am J Nephrol* 11:98–101, 1991.
21. Huang SN, Neurath AR: Immunohistologic demonstration of hepatitis B viral antigens in liver with reference to its significance in liver injury. *Lab Invest* 40:1–17, 1979.
22. Rugge M, Guido M, Bortolotti F, Cassarom M, Cadrobbo P, Noventa F, Realdi G: Histology and virus expression in the liver: A prognostic puzzle in chronic hepatitis B. *Virchows Archiv A* 419:93–97, 1991.
23. Gudat F, Bianchi L, Sonnabend W, Thiel G, Aenishaenslin W, Stalder GA: Pattern of core and surface expression in liver tissue reflects state of specific immune response in hepatitis B. *Lab Invest* 32:1–9, 1975.
24. Consolo F, Freni MA: Nosography and immunopathogenesis of viral hepatitis. *Nephron* 61:251–254, 1992.
25. Hammerer P, Kramer-Hansen H, Kremer B, Henne-Bruns D, Kloppel G, Arnot R, Huland H: Aspiration cytology of liver transplants. *Transplant Proc* 20:640–641, 1988.
26. Cohen GA, Goffinet JA, et al: Observations on decreased serum glutamic oxalacetic transaminase (SGOT) activity in azotemic patients. *Ann Int Med* 84:275–280, 1976.

27. Vaziri ND, Kim I: Serum enzyme levels. In *Handbook of Dialysis* (Daugirdes JT, Ing TS, eds). Boston: Little, Brown, 1988, pp 316–320.
28. Mosconi G, Campieri C, Miniero R, Coli L, Orsi C, La Manna G, De Sanctis LB, Stefoni S, Sprovieri G, Bonomini V: Epidemiology of hepatitis C in a population of hemodialysis patients. *Nephron* 61:298–299, 1992.