SHORT COMMUNICATION

Microalbuminuria in Chronic Hepatitis B Infection

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Received: October 18, 2008;
Initial review: March 30, 2009;
Accepted: May 11, 2009.

We examined for microalbuminuria in patients with hepatitis B virus (HBV) infection, and the effect of antiviral treatment. Group I consisted of 38 patients who were inactive HbsAg carriers; group II included 21 HBeAg positive patients with chronic HBV infection who responded to antiviral treatment at 6 months; group III consisted of 24 patients with chronic HBV infection who did not respond to treatment at the end of 6 months; and group IV consisted of healthy controls. Initial level of microalbuminuria was significantly higher in group II compared to the levels measured at 3, 6, and 9 months (P<0.001). Although, there was a significant difference in microalbuminuria at initial and 3 months between group I and group II (P<0.001), no differences were found at 6 and 9 months. There was no significant difference betweeen group II and group III in terms of urine microalbuminuria at the beginning of the study, but statistically significant differences were determined at 3, 6, and 9 months (P<0.001). The measurement of microalbuminuria may indicate a preclinical renal damage, associated with chronic HBV infection. It may also be used to determine the response to treatment with interferon and lamivudine in children with HBV infection.

Keywords: Children, Hepatitis B, Microalbuminuria, Renal damage, Treatment.

Published online: 2009 September 03. PII: S097475590800615-2

Chronic HBV infection has been reported to be associated with several types of glomerulonephritis (GN), including membranous glomerulopathy, membranoproliferative GN, mesangial proliferative GN, minimal change disease, focal glomerulosclerosis, and IgA nephropathy. The most common type of GN is membranous glomerulopathy, which has generally a benign course in children(1-3).

The role of the immune complexes has been examined in HBV infection. In acute and chronic HBV infections, immune complexes formed by antibodies against surface (HbsAg) and envelope (HbeAg) antigens and autoantibodies formed during liver damage may play a significant role in pathogenesis(4). These circulating immune complexes may be deposited in the kidneys resulting in GN. Patients with chronic HBV infection show deposition of HBsAg, antiHBs antibodies and complement 3 (C3) in the glomerular basal membrane(4,5). Proteinuria is an important indicator of renal disease. The presence of microalbuminuria suggests the presence of glomerular damage in patients with diabetes mellitus (diabetic nephropathy)(6).

This study was planned to examine for microalbuminuria as an indicator of glomerular damage in patients with HBV infections. We also examined the relationship between microalbuminuria and the clinical and laboratory findings at different stages of HBV infection in patients receiving medical treatment.

METHODS

Patients with chronic HBV infections followed prospectively at the Pediatric Clinic of Firat University Hospital between January 2003 and July 2005 were classified into four groups. Group I

consisted of 38 patients who were inactive HbsAg carriers; group II consisted of 21 HBeAg positive patients with chronic HBV infection who received treatment (alpha interferon and lamivudine) and responded to treatment at 6 months; and group III consisted of 24 patients with chronic HBV infection, who did not respond to treatment (alpha interferon and lamivudine) at 6 months and the treatment was extended to 12 months. Control subjects were healthy children with no abnormality on physical examination and laboratory tests (group IV). All patients had normal renal functions, glucose levels, and normal blood pressure and used no medications. Patients with systemic diseases (such as diabetes mellitus, hypertension, nephrotic syndrome, amyloidosis and rheumatologic diseases) along with HBV infection were excluded from the study. Patients positive for HbsAg and antiHBe, and negative for antiHBs, HBeAg, HBV DNA and normal liver enzymes for 6 or more months were defined as inactive HBsAg carriers (group I); patients with high levels of HBsAg, HBeAg, and HBV DNA, and negative for antiHBs, antiHBe, and increased liver enzymes for 6 or more months were considered to have chronic HBV infection (groups II and III).

Patients in group I were followed without treatment. Patients in groups II and III were treated with a dose of 5 million U/m^2 of alpha-interferon (α-INF) administered subcutaneously three times a week and lamivudine at a dose of 4 mg/kg/day(7,8). HBeAg seroconversion (HBeAg negative, antiHBe positive) and decrease in the level of serum transaminases and HBV DNA titers were considered as the criteria for response to treatment in patients receiving treatment. Patients in group II were considered as responders at the end of 6 months based on the response criteria and the treatment was discontinued. Treatment was extended to 12 months in group III patients when there was no improvement at the end of 6 months of treatment(7-9).

Microalbumin excretion was measured in 24 h urine collected 4 times in group I, II and III patients at the beginning of the study and every 3 months. Complete blood count, urinalysis, complement 3 (C3), protrombin time, HBV serology and HBV DNA titers were examined at each visit. In the control group, 24 h urine microalbumin excretion was estimated thrice. HBV serological markers were studied by commercial ELISA kits (Tecan Genesis, Zurich). Quantification of HBV DNA was done by a sandwich nucleic acid hybridization assay(10). Urine microalbuminuria levels were estimated with the immunotrubidimetric method (Cobas Integra 800, Germany) using commercial kits.

Statistical analyses was performed using SPSS 11.0 (Chicago, Illinois, USA). Data was expressed as mean ± standard deviation. Variance analysis, one-way ANOVA and Tukey test were used in order to evaluate the differences between the groups.

RESULTS

Of the 116 patients, 65 (56%) were boys; the groups were similar with regard to age. The baseline and important laboratory features of patients were showed ***Table* I.** There was no change in levels of alanine aminotransferase (ALT), aspartate amino-

TABLE I BASELINE FEATURES OF THE STUDY POPULATION

	Group I (*n*=38)	Group II (*n*=21)	Group III (*n*=24)	Group IV (*n*=33)
Age, y	7.7 ± 3.5	7.5 ± 3.2	7.9 ± 3.6	8.1 ± 2.5
Female/male	15/23	10/11	9/15	17/16
Urea nitrogen, mg/dL	25.4 ± 7.1	28.9 ±10.6	25.0 ± 6.5	31.8 ± 9.9
Blood creatinine, mg/dL	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
Total protein, g/dL	7.4 ± 0.5	7.4 ± 0.5	7.5 ± 0.5	7.2 ± 0.4
Albumin, g/dL	4.4 ± 0.3	4.4 ± 0.3	4.4 ± 0.3	4.4 ± 0.2
Prothrombin time, sec	13.4 ± 1.9	13.9 ± 2.2	13.4 ± 2.1	14.4 ± 2.0

Values represent mean ± standard deviation.

transferase (AST), and HBV DNA of patients in group I during 9 months follow up ($P>0.05$). Three patients in group I (one at 3 months and 2 at 6 months) were lost to follow up. In group II, the ALT, AST, HBV DNA and microalbuminuria levels measured at the beginning of the study were significantly increased compared to values at 3, 6 and 9 months ($P<0.001$). Two patients were lost to follow up at the end of the 3 months in this group.

There was no statistically significant change in levels of ALT, AST and HBV DNA in patients in group III at 9 months follow up ($P>0.05$). In group III, one patient was lost to follow up at 3 months and two patients after 6 months. However, the microalbuminuria and serum ALT, AST, HBV DNA levels of these were also increased. The serum ALT, AST, HBV DNA and microalbuminuria values of all groups at the beginning and at 3, 6 and 9 months are shown in ***Table* II**.

While comparison within groups I and II in terms of serum ALT, AST and microalbuminuria levels showed a statistically significant difference at the beginning and at 3 months ($P<0.001$), there were no differences at 6 and 9 months. Serum ALT, AST and microalbuminuria levels were significantly increased in patients in group III when compared to these in group I ($P<0.001$). No significant difference was present between group I and the controls in respect to

TABLE II LEVELS OF SERUM TRANSAMINASES, HBV DNA AND MICROALBUMINURIA

	Group I (*n*=38)	Group II (*n*=21)	Group III (*n*=24)	Group IV (*n*=33)	*P*
ALT (U/L)					
Baseline	41.8 ± 15.5	147.6 ± 41.9	179.0 ± 70.5	40.6 ± 12.3	<0.001 (Group I-II, I-III, II-IV, III-IV)
3-months	35.6 ± 14.1	73.4 ± 21.4	164.7 ± 54.6	–	<0.001(Group I-II, I-III, II-III)
6-months	46.4 ± 17.4	41.0 ± 10.2	165.3 ± 51.2	–	<0.001 (Group I-III, II-III)
9-months	53.1 ± 13.8	42.3 ± 10.1	177.9 ± 49.7	–	<0.001 (Group I-III, II-III)
AST (U/L)					
Baseline	51.6 ± 19.0	151.1 ± 71.3	156.6 ± 52,9	38.2 ± 12.8	<0.001 (Group I-II, I-III, II-IV, III-IV)
3-months	50.1 ± 14.7	83.5 ± 17.2	163.4 ± 61.4	–	<0.001(Group I-II, I-III, II-III)
6-months	51.3 ± 18.0	41.6 ± 15.0	177.0 ± 52.8	–	<0.001 (Group I-III, II-III)
9-months	51.5 ± 12.5	41.5 ± 13.7	168.4 ± 55.6	–	<0.001 (Group I-III, II-III)
HBV/DNA (pg/mL)					
Baseline	–	671.5 ± 382.0	694.7 ± 304.5	–	<0.001 (Goup I-II, I-III)
3-months	–	39.5 ± 19.6	657.4 ± 304.5	–	<0001 (Group I-II, I-III, II-III)
6-months	–	7.9 ± 2.9	600.2 ± 259.5	–	<0.001 (Group I-III, II-III)
9-months	–	5.0 ± 2.7	634.2 ± 284.8	–	<0.001 (Group I-III, II-III)
Microalbuminuria (mg/24 h)					
Baseline	8.9 ± 3.5	46.2 ± 17.2	40.8 ± 18.0	6.4 ± 3.2	<0.001 (Goup I-II, I-III)
3-months	10.4 ± 2.9	20.3 ± 11.0	43.2 ± 17.8	8.9 ± 3.7	<0.001 (Group I-II, I-III, II-III)
6-months	11.4 ± 3.0	10.5 ± 4.1	38.7 ± 15.1	7.0 ± 2.5	<0.001 (Group I-III, II-III)
9-months	9.9 ± 3.6	7.7 ± 3.0	41.6 ± 15.8	–	<0.001 (Group I-III, II-III)
Complement, C3(g/L)					
Baseline	–	0.73 ± 0.21	0.74 ± 0.23	–	>0.05 (Group II-III)
6-months	–	1.30 ± 0.32	0.76 ± 0.24	–	<0.05 (Group II-III)

ALT: alanine aminotransferase; AST: aspartate aminotransferase; HBV DNA: Hepatitis B virus DNA.

WHAT THIS STUDY ADDS?

- Monitoring microalbuminuria provides information about the response to treatment and determines the preclinical renal damage in children with chronic hepatitis B infection.

microalbuminuria during the study ($P<0.05$). While there were no differences between group II and group III at the beginning of the study in terms of ALT, AST, HBV DNA and micro-albuminuria levels ($P>0.05$), a statistically signi-ficant differences were determined at 3,6 and 9 months ($P<0.05$). Patients in group III showed significantly lower levels of C3 at 6 months compared to patients in group II (***Table* II**).

DISCUSSION

Proteinuria is one of the most common symptoms of kidney diseases and may be frequently detected in patients in the preclinical period. Increasing micro-albuminuria levels indicate glomerular damage. Persistently increased levels of microalbuminuria more than 30 mg/day in diabetic patients are considered as an indicator of diabetic nephro-pathy(11,12). Similarly, it has been reported that microalbuminuria over 15 mg/day in children with recurring acute tonsillitis and tonsillar hypertrophy may be a sign of renal damage(13). Micro-albuminuria has also been used in the follow up of patients with poststreptococcal GN(14). To our knowledge, there is no study evaluating the role of microalbuminuria during the follow up of children with chronic HBV infection and its role in determining the response to treatment.

GN associated with chronic HBV infection develops due to immune reaction caused by the deposition of immune complexes in the glomerular basal membrane. Histopathological appearance is similar to poststreptoccocal, membranous or membranoproliferative GN. Furthermore, the level of C3 decreases in GN associated with chronic HBV infection. The clinical picture subsequently improves but the course of renal deficiency is not defined(5,15). In our study, the C3 level was low in groups II and III at the beginning and then normalized, along with the reduction of the microalbuminuria level at 6 months in group II. This may be an indicator of a reduction in virus load, immune complex deposition and an improvement in the glomerular damage. In group III, C3 level was still lower after 6 months. However, the fact that the microalbuminuria levels were low in three patients in group II and four patients in group III since the beginning suggests that there may be different immune responses in the patients. High micro-albuminuria levels and low serum C3 levels were determined in patients with chronic active hepatitis in our study. Low C3 levels may be due to both production deficiency and activation of complement system by antigen-antibody and immune complexes in subjects with liver diseases. We speculate that low C3 is probably due to excessive consumption resulting from complement activation.

In our study, ALT, AST and microalbuminuria levels were normal in group I. We hypothesized that normal levels of microalbuminuria may be due to lower virus load (absence of virus replication indicators) and the absence of renal damage caused by immune complex. However, positive HBeAg and high HBV DNA levels in groups II and III demonstrate active virus replication. It may be assumed that the glomerular damage due to immune complex deposition during active infection leads to an increase in microalbuminuria levels. Reduction in levels of microalbuminuria in group II following treatment and persistent microalbuminuria in patients in group III, support this view.

In conclusion, measurement of microalbu-minuria in urine, which is a cost-effective and noninvasive method, may be a sign of preclinical renal damage that may develop in chronic HBV infection and an indication of response to treatment. Further studies, that incorporate estimation of blood levels of circulating immune complexes and renal histology, are necessary to confirm our findings.

Contributors: MK conceived and designed the study and revised the manuscript for important intellectual content. He will act as guarantor of the study. MK and ET collected

data and drafted the paper. ET and YD conducted the laboratory tests, and interpreted them. YS analyzed the data and helped in manuscript writing. The final manuscript was approved by all the authors.

Funding: None.

Competing interests: None stated.

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