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Original Article

Hepatitis B Virus-Related Glomerulopathy in Egyptian Children

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Abstract

Objectives: To study hepatitis B virus (HBV) markers (HBsAg, HBeAg and anti-HBc antibody) together with the circulating immune complexes (CIC), C3 and liver function tests in children with renal diseases.

Methods: Subjects studied were, 20 healthy children as a control group, 20 patients with acute glomerulonephritis. (AGN) and 50 patients with nephrotic syndrome (NS), 25 during relapse and 25 patients during remission.

Results: The incidence of HBsAg and HBeAg was insignificantly different in patients with AGN and in nephrotic patients in comparison with the control group. However, the incidence of anti-HBc antibody was significantly higher in patients with AGN (30%) and in nephrotic patient during relapse (44%) compared to healthy children. There was a significant increase in serum creatinine, SGPT, SGOT, alkaline phosphatase, serum bilirubin and CIC with a significant decrease in serum albumin and C3 in AGN patients with positive anti-HBc antibody in comparison to Anti-HBc negative patients.

In nephrotic patients during relapse, the incidence of frequent relapses was significantly higher in anti-HBc antibody positive patients, and the serum SGPT, SGOT, alkaline phosphatase, bilirubin, CIC were significantly high and C3 was significantly low in comparison with Anti-HBc antibody negative patients.

Renal biopsy was performed in 4 anti-HBc antibody positive nephrotic patients and showed membranoproliferative glomerulonephritis (2 cases), membranous glomerulonephritis (1 case) and focal glomerulosclerosis (1 case).

Conclusions: From these results we suggest that infection with HBV may be one of the aetiological factors of AGN and NS in Egypt. Recognition of secondary glomerulonephritis has significant prognostic and therapeutic implications.

INTRODUCTION

The association between HBV infection and glomerular disease was first reported by Combes and coworkers, 1971[1]. HBV has been implicated in the pathogenesis of AGN[2], and of NS[3]. The glomerular lesion appears to be initiated by deposition of circulating immune-complexes. HBV DNA, HBe Ag-antibody immune complexes and IgG were detected by in-situ hybridization in the glomeruli of 87.5% of patients with HBV associated glomerulonephritis (HBVGN)[4]. Also circulating immune complexes (CIC) containing HBsAg and HBeAg were detected in the sera of 5 out of 6 children with HBVGN[5].

The incidence of HBV-related nephropathy depends on the local prevalence of HBsAg. Chang and Yeoh (1985)[6], suggested that, with a local prevalence of HBsAg carriers, as high as 9.5%, a study to clarify the pathogenetic role of HBV in the development of glomerulonephritis is needed.

The management and prognosis of HBVGN may be different from that of

idiopathic MGN. It was found that steroids are of no benefit in the treatment of HBVGN[7]. On the other hand, Lin (1991)[8] suggested that steroids may lead to a potential risk of enhancing viral replication. Although not always successful, interferon treatment should be considered in severe persistent nephrotic states associated with HBV[9].

Owing to the high incidence of HBs antigenemia (8.6%) reported in Egypt[10], this study was performed to evaluate the role of HBV in the pathogenesis of glomerulopathy in Egyptian children.

SUBJECTS AND METHODS

Ninety children, aged from 3 - 14 years, were included in this study. They were mainly from the pediatric nephrology clinic of Ain Shams University, and some were in-patients in the same hospital and in El Sahel Hospital. The studied children were categorized into 4 groups:

Group I (control group): comprised 20 healthy children (8 boys and 12 girls), aged from 3 -11 years.

Group II: included 20 patients with AGN (9 boys and 11 girls), aged from 3 - 11 years. Duration of illness ranged from 2 - 15 days. All the patients presented with oliguria, hematuria (smoky or frank), oedema and hypertension. Only one case had previous history of jaundice 4 weeks before the onset of clinical manifestations of AGN.

Group III: included 25 patients with NS during relapse (18 boys and 7 girls), aged from 3 - 14 years. They presented with generalized oedema, heavy proteinuria (> 2 gm/24 hour urine), hypoproteinemia and hypercholesterolemia. Ascites was found in most of the cases. The duration of their illness ranged from 10 months to 7 years. Four were steroid resistant, 8 were frequent relapsers, and 13 were infrequent relapsers.

Group IV: included 25 patients with NS during remission (16 boys and 9 girls), aged from 4 - 14 years. There was no proteinuria or oedema in these patients. Duration of remission was at least 6 months. The duration of their illness ranged from 10 months to 11 years. Twenty were infrequent relapsers and the remaining five children were frequent relapsers.

The Laboratory Tests Included:

- Complete urine analysis.
- Estimation of blood urea, serum creatinine, total serum proteins, serum albumin, serum cholesterol, serum bilirubin, SGPT, SGOT and alkaline phosphatase.
- Measurement of CIC, using kits supplied by Dia Medical corporation, 2140 North Miami Avenue, Florida 33127.
- Determination of C3 by single radial immunodiffusion using commercially available kits "Behring".
- Detection of HBsAg by non-competitive enzyme immunoassay, HBeAg by direct, non-competitive enzyme linked immunosorbent assay and Anti-HBc antibody by a competitive enzyme immuno-assay, using kits supplied by Sorin Biomedica 13040, Saluggia (VC) Italy.

RESULTS

The results of the present work are summarized in Tables (1-3).

As regards patients with AGN, hepatomegaly was found in 5 (4 of them were Anti-HBc antibody + ve) and splenomegaly in 1 patient with positive Anti-HBc antibody. Ascites and jaundice were not found. HBsAg, HBeAg and Anti HBc antibody were positive in one patient who had past history of jaundice 4 weeks before the onset of nephritis. In this patient, CIC was 82 μEq/ml and C3 was 0. Anti-HBc antibody was positive in 6 patients (30%) and this was significantly high compared with the normal controls ($p < 0.05$). CIC were significantly increased (38.2 $\pm$ 18 uq Eq/ml) and C3 was significantly decreased (48.1 $\pm$ 24.8) ($p < 0.001$) (Table 1). CIC more or equal to twenty μEq/ml was considered abnormally high while CIC less than twenty was considered normal. CIC was high in 19 patients and C3 was depressed in all the patients studied.

Table (2) shows that serum creatinine, SGPT, SGOT, alkaline phosphatase, serum bilirubin and CIC were significantly increased, while serum albumin and C3 were significantly decreased in anti-HBc antibody positive AGN patients in comparison with anti-HBc antibody negative nephritic patients and to the control group. SGPT and SGOT were more than double the normal values in two patients positive for anti-HBc antibody. Liver enzymes were normal in all the patients who were negative for anti-HBc antibody.

As regards nephrotic patients during relapse, hepatomegaly was found in 11 cases, splenomegaly in 3 cases and ascites in 12 patients. Mean systolic blood pressure was 103 $\pm$ 12 mm Hg and the diastolic pressure was 65 $\pm$ 9.9 mm Hg. The prevalence of HBsAg was 16% (4 patients) and HBeAg was 8% (2 patients) and these were insignificantly different from the control group ($p > 0.05$). Anti-HBc antibody was positive in 11 cases (44%) and this was significantly high in comparison with the control group. Hepatomegaly was found in 10 of them. Among these 11 patients, four were steroid resistant, six were frequent relapsers and one was infrequent relapser. CIC were significantly increased ($p < 0.001$.) and C3 was significantly decreased ($p < 0.05$) when compared to healthy children (Table 1). CIC were high in 17 patients and C3 was depressed in 15 patients. SGPT and SGOT were more than double the normal value in three patients and were more than one and one half times the normal in two patients.

Table (3) shows that SGPT, SGOT, alkaline phosphatase, serum bilirubin and CIC were significantly increased and C3 was significantly decreased in anti-HBc antibody positive nephrotic patients in relapse compared to anti-HBc antibody negative patients and the control group. There was a high incidence of frequent relapses in Anti-HBc antibody positive nephrotic patients in relapse in comparison with Anti-HBc antibody negative patients ($p < 0.001$).

Renal biopsy was done in 4 anti-HBc antibody positive steroid resistant nephrotic patients and revealed membranoproliferative glomerulonephritis (MPGN, 2 cases), membranous glomerulonephritis (MGN, 1 case) and focal glomerulosclerosis (FGS, 1 case). Immunofluorescent study was performed in

2 cases and showed immune-complex deposition.

HBsAg and HBeAg were not detected in nephrotic patients in remission and anti-HBc antibody was positive in 4 cases (16%), and this was insignificantly different from the control group. Hepatomegaly was found in 3 patients (12%), 2 of them were Anti-HBc antibody positive. CIC and C3 were also insignificantly different from the control group (Table 1). SGPT and SGOT were increased in three patients (more than one and one half times the normal levels).

Table (1): Hepatitis B markers, CIC and C3 in all the studied groups

	HBsAg	HBeAg	Anti-HBc	CIC	C3	Hepatomegaly
AGN (20)	1	1	6	$28.2 \pm 18^{***}$	$48.1 \pm 24.8^{***}$	5
NS during relapse (25)	4	2	11	$41 \pm 25.9^{**}$	$80.3 \pm 55.1^{*}$	11
NS during remission (25)	0	0	4	24.5 ± 16.3	116.3 ± 60.9	3
Controls (20)	1	0	1	7.5 ± 5.4	130.8 ± 28.9	0

p values of the studied patients versus controls are denoted as:
* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Table (2): Comparison between the biochemical parameters, CIC and C3 of anti-HBc positive and negative AGN patients and the control group

	AGN Patients		
	Anti-HBc +ve	Anti-HBc –ve	Control
	n = 6	n = 14	n = 20
Blood urea (mg/dL)	$110.8 \pm 30^{***}$	$91.4 \pm 23.9^{***}$	25 ± 3.2
Serum creatinine (mg/dL)	$1.66 \pm 0.25xx,^{***}$	$1.2 \pm 0.2^{***}$	0.78 ± 0.8
Serum cholesterol (mg/dL)	159.1 ± 11	154.6 ± 11	156.5 ± 16.1
Total serum proteins (gm/dL)	6.83 ± 0.67	7.04 ± 0.5	7.2 ± 0.3
Serum albumin (gm/dL)	$3.05 \pm 0.82xx,^{**}$	4.3 ± 0.6	4.2 ± 0.2
SGPT (μ/L)	$54.1 \pm 30.9xx,^{**}$	10 ± 2.7	10.0 ± 2.9
SGOT (μ//L)	$79.6 \pm 47.2xx,^{**}$	10.5 ± 3.7	11.8 ± 2.9
Alkaline phosphatase (K.A.U.)	$18.3 \pm 6xx,^{**}$	11.1 ± 1.09	11.4 ± 0.8
Serum bilirubin (mg/dL)	$0.86 \pm 0.2xxx,^{***}$	0.4 ± 0.13	0.41 ± 0.15
CIC (μ/Eq/ml)	$54.5 \pm 20.8x,^{***}$	$31.1 \pm 11.5^{***}$	7.5 ± 5.4
C3 (mg/dL)	$21.0 \pm 19.5x,^{***}$	$52.8 \pm 25.5^{**}$	130.8 ± 28.9

- All the values are presented as the mean ± S.D.
- p values of anti-HBc positive AGN patients versus anti-HBc negative patients are denoted:
  x $p < 0.05$ xx $p < 0.01$ xxx $p < 0.001$
- p values of AGN patients (anti-HBc +ve or –ve) versus the control group are denoted:
  * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Table (3): Comparison between the biochemical parameters, CIC and C3 of anti-HBc positive and negative nephrotic patients during relapse and the control group

	Nephrotic Patients		Control
	Anti-HBc +ve	Anti-HBc –ve	
	n = 6	n = 14	n = 20
Blood urea (mg/dL)	54.9 ± 28.5**	44.2 ± 17.3**	25 ± 3.2
Serum creatinine (mg/dL)	0.9 ± 0.2**	0.85 ± 0.3	0.73 ± 0.08
Serum cholesterol (mg/dL)	748 ± 128***	657 ± 148***	156.5 ± 16.1
Total serum proteins (gm/dL)	4.5 ± 0.7***	4.7 ± 0.8***	7.2 ± 0.3
Serum albumin (gm/dL)	1.6 ± 0.4***	1.5 ± 0.4***	4.2 ± 0.2
SGPT (μ/L)	49.7 ± 29.6xxx,**	9.9 ± 2.8	10 ± 2.9
SGOT (μ//L)	60.6 ± 41.7xxx,**	11.6 ± 3.6	11.8 ± 2.9
Alkaline phosphatase (K.A.U.)	14.4 ± 1.2xxx,***	12 ± 0.8	11.4 ± 0.8
Serum bilirubin (mg/dL)	0.6 ± 0.19xx,**	0.4 ± 0.1	0.41 ± 0.15
CIC (μ/Eq/ml)	65.6 ± 14.6xxx,***	21.9 ± 13.6	7.5 ± 5.4
C3 (mg/dL)	28.0 ± 24.1xxx,***	120.5 ± 33.2	130.8 ± 28.9

- All the values are presented as the mean ± S.D.
- p values of anti-HBc positive nephrotic patients versus anti-HBc negative patients during relapse are denoted:
  x $p < 0.05$ xx $p < 0.01$ xxx $p < 0.001$
- p values of nephrotic patients during relapse (anti-HBc +ve or –ve) versus the control group are denoted:
  * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

DISCUSSION

Transient glomerulonephritis accompanying acute viral hepatitis has been noted previously[11]. In chronic active hepatitis, coincident glomerulonephritis has been found in as many as 50% of the cases studied by Mistilis and Blackburn (1970)[12]. Also symptom - free HBsAg carriers, should be regarded as being at risk of developing glomerulonephritis[2].

In the 70 children with glomerulopathy studied, the incidence of HBsAg and HBeAg were not significantly increased, however the incidence of anti-HBc antibody was significantly increased in AGN patients (30%) and in nephrotic patients during relapse (44%) in comparison with the control group. Also C3 was significantly decreased and CIC were significantly increased in AGN and in nephrotic patients during relapse. However during remission anti-HBc antibody, C3 and CIC were not significantly different from the normal controls.

Anti-HBc antibody is not a virus neutralizing antibody and therefore, in contrast to anti-HBs antibody is not protective[13]. It is the single most reliable marker of HBV infection but is not useful in determining the stage or duration of the disease. It indicates active HBV infection (acute and chronic) as it persists as long as viral replication within the liver cells continues[14]. The significant increase of Anti-HBc antibody with no significant increase of HBsAg in our patients

shows that these children may be in the window period during recovery from acute infection or this profile represents recovery from distant past infection. In individuals with the last pattern HBsAg has declined to levels below those detectable by immunofluorescence[15].

The significantly high incidence of Anti-HBc antibody (30%) in AGN patients together with hepatomegaly (5 cases) and the impaired liver functions may indicate that infection with HBV (whether concurrent or past) may be the cause of nephritis in these patients, although all of them (except one) had no history of jaundice prior to the onset of renal trouble. One patient had nephritis 4 weeks after acute hepatitis and was positive for HBsAg, HBeAg and anti-HBc antibody together with markedly increased CIC and zero level of C3. Anti-HBc antibody positive AGN patients had significantly elevated levels of serum creatinine, SGOT, SGPT, alkaline phosphatase, serum bilirubin, CIC and significantly decreased serum albumin and C3 in comparison with those negative for Anti-HBc antibody. These findings agree with the suggestion of Kar et al (1987)[16] in Japan, that HBV antigenemia plays a significant role in the development of specific forms of glomerulonephritis. They detected HBsAg in 69 of 311 patients (22%) with primary glomerulonephritis and this was significantly higher than in the general population.

The significantly low levels of C3 (15 patients out of 25) and the elevated levels of CIC (in 21 patients) found in nephrotic patients during relapse, suggest immune complex disease and not the minimal change type. Most probably the antigen in these cases was HBV, evidenced by the high incidence of anti-HBc antibody (44%), hepatomegaly (found in 11 cases), impaired liver function tests and finally by the immunofluorescent study of 2 renal biopsy specimens which revealed immune complex deposition. Previous reports of a relationship between hypocomplementemia and HBVGN are contradictory. Kleinkecht et al. (1979)[17] found that 14 of 15 French children with HBVGN had normal C3 values, whereas the South West Pediatric Nephrology Study Group (1984)[18] found hypocomplementemia in all 11 children studied. A relationship between complement activation and the activity of renal disease is also suggested by the finding of normal C3 levels among patients in remission.

The significantly elevated anti-HBc antibody in nephrotic patients during relapse (44%) compared with the normal controls may suggest that infection with HBV may be responsible for the development of nephrotic syndrome in these patients. On the other hand the depressed immunity encountered in these children may be a predisposing factor to increased incidence of infection. The association of nephrotic syndrome and HBV infection increases the severity of the renal trouble, evidenced by the high incidence of frequent relapses in anti-HBc antibody positive patients compared with anti-HBc antibody -ve patients. HBVGN in children is typically membranous or less commonly MPGN.[19] Hepatitis B virus (HBV) infection is recognized as an important cause of nephrotic syndrome in endemic areas[7].

The significant role of HBV infection in the pathogenesis of nephrotic syndrome and glomerulonephritis was previously reported by Brzosko et al. (1974)[2] in Poland. They found immunoglobulins and complement by immunofluorescence in 32 of 52 unselected kidney biopsy specimens from children with clinical nerphrosis and or glomerulo-nephritis. Deposits with the composition and characteristics of HBsAg/antibody complexes were identified in 18 of these 32 specimens (56.2%). HBsAg was detected by immunoelectroosmophoresis in the sera of 16 of these patients and Anti-HBc antibody was detected by indirect immunofluorescence in all 18 cases.

In our nephrotic patients during remission, all HBV markers studied as well as CIC and C3 were not significantly different from the control group. This means that the occurrence of clinical remission is more common in patients with no evidence of infection with HBV.

The favorable clinical evolution and seroconversion of HBeAg and HBsAg was previously reported by Yamashita et al. (1985)[20].

Renal biopsy was done in 4 patients during relapse with +ve HBsAg and revealed MPGN (2 cases), MGN with mesangial proliferation (one case) and FGS (one case). Immunofluorescent study was performed on 2 cases and showed deposits of IgG and IgM. The deposits of immunoglobulins in renal biopsy studied suggest that the pathogenesis of N.S. in these cases is most probably due to immune complexes deposition. Membranous nephropathy[21] and MPGN[22] have been reported as common pathologic features of HBV-related glomerulopathy in children.

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