SEROLOGICAL PATTERN OF HEPATITIS B VIRUS MARKERS (HBsAg, ANTI-HBs, IgM ANTI-HBc AND HBV SPECIFIC DNA POLYMERASE) IN LEPROSY PATIENTS

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ABSTRACT : *Sera of* 134 *lepromatous (LL/BL) and* 57 *tuberculoid (TT/BT) leprosy patients were analysed for four HBV markers. HBsAg was detected in* 6.71% *of lepromatous and* 3.5% *of tuberculoid sera. The per cent positivity of lepromatous and tuberculoid sera for anti-HBs antibodies was* 30.59% *and* 35.08%, *respectively. The positivity of normal sera for HBsAg and anti-HBs was* 3.60% *and* 21.69%, *respectively. The difference in the positivity of three groups of sera (lepromatous, tuberculoid and normal) for HBsAg or anti-HBs was not statistically significant. Anti-HBc (IgM) antibodies were detected in* 6% *of lepromatous sera. HBV-specific DNA-polymerase activity was found in* 22.22% *of HBsAg positive (but anti-HBc negative) sera, and* 66.66% *of anti-HBc positive (but HBsAg negative) sera. The pattern of acute HBV infection in leprosy patients followed the typical pattern prevalent in the normal population.*

INTRODUCTION

A high prevalence of hepatitis B surface antigen (HBsAg) in patients with lepromatous leprosy (LL) was first reported by Blumberg *et al* (1967). This was followed by several reports from different parts of the world. Several investigators reported an increased prevalence of HBsAg in LL patients (Blumberg and Melartin, 1970; Fakunle and Whittle, 1981; Kelkar *et al*, 1974; Papaevangelou *et al*, 1972; and Saha and Dutta, 1977). The increased prevalence of HBsAg in LL patients was attributed to either the genetic susceptibility (Blumberg *et al*, 1967; Serjeantson and Woodfield, 1978), or the impaired cell mediated immune response (Fakunle and Whittle, 1981; Saha and Dutta, 1977) of the leprosy patients. However, in contrast to the above studies, other investigators found no increase in the prevalence of HBsAg in LL patients as compared to that in patients with tuberculoid leprosy (TT) or normal healthy individuals (Lechat *et al*, 1973;

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Salzano and Blumberg, 1970; Sher *et al*, 1977; Shwe and Zuckerman, 1972; Papageorgiou *et al*, 1972; and Swanepoel and Cruickshank, 1972). The results of these studies suggested that the prevalence of HBsAg in leprosy patients simply followed the pattern prevalent in the healthy population in that environment.

In view of the conflicting observations of different studies it is difficult to draw a clear cut conclusion regarding increased susceptibility of LL patients to hepatitis B virus (HBV) infection and the persistence of HBsAg. A major limitation of most of the previous studies was that the whole emphasis was only on one parameter, viz. HBsAg, and the techniques employed for its detection in serum (e.g. double diffusion in agar or counter-immunoelectrophoresis) were less sensitive than modern techniques like enzyme immunoassays (EIAs) or radioimmunoassays (RIAs). In addition, there are only few studies in which anti-HBs antibodies (antibodies to HBsAg) have been studied alongwith HBsAg. The present study is aimed at evaluating the association between HBV infection and leprosy using four HBV markers i.e., HBsAg, anti-HBs antibodies, anti-HBc (IgM) antibodies, and DNA-polymerase, all determined by sensitive assays. To avoid the effect of institutionalization on any one category of patients, the study was performed on leprosy patients who were being managed as outpatients.

MATERIALS AND METHODS

Patient sera : Sera were collected from 134 lepromatous (LL/BL) and 57 tuberculoid (TT/BT) patients, attending the outpatient leprosy clinic at Safdarjang Hospital, New Delhi. The patients were classified according to the Ridley and Jopling (1966) classification. Sera were stored at —70°C till analysed.

Normal sera : Sera were also collected from 4000 young adults (males and females) from a rural area where the chances of viral infection due to needle stick, post-transfusion or post-instrumentation are very rare.

HBV-markers : HBsAg was tested by micro ELISA test (Gandhi and Tandon, 1984). IgM antibodies to the core antigen of HBV (anti-HBc) and anti-HBs antibodies were analysed by EIA kits from Abbott Laboratories, USA. HBV-specific DNA polymerase (DNA-P) activity was tested by the method of Fang *et al* (1981). Serum transaminases were tested by standard biochemical methods.

Statistical analysis : Statistical analysis was done by using the test of proportion.

RESULTS

Sera of 191 leprosy patients, including 134 lepromatous (LL/BL) and 57 tuberculoid (TT/BT) patients, were analysed for four HBV markets, namely HBsAg, anti-HBs antibodies, IgM anti-HBc antibodies and HBV specific DNA-polymerase. The results are summarized in Tables 1 and 2. HBsAg was detected in 9 out of 134 (6.71%) lepromatous, and 2 out of 57 (3.5%) tuberculoid sera. The per cent positivity of lepromatous sera for anti-HBs antibodies was 30.59% in comparison to 35.08%

TABLE : 1 **The positivity of leprosy sera for different HBV-markers.**

GROUP	NUMBER POSITIVE/TOTAL SERA AND (%) POSITIVITY FOR		
	HBsAg	ANTI-HBc (IgM) ANTIBODIES	ANTI-HBs ANTIBODIES
1. Lepromatous (LL/BL) Leprosy	9/134(6.71%)	8/134(5.97%)	41/134(30.59%)
2. Tuberculoid (TT/BT) Leprosy	2/57(3.50%)	0/57(—)	20/57 (35.08%)
3. Normal controls	144/4000(3.60%)	—	64/295(21.69%)

TABLE 2 : **HBV-specific DNA Polymerase activity in leprosy sera**

GROUP	NUMBER POSITIVE/TOTAL SERA AND (%) POSITIVITY
I. HBsAg positive (but anti-HBc negative)	
1. LL/BL	2/6 (33.33%)
2. TT/BT	0/3 (—)
3. Total	2/9 (22.22%)
II. Anti-HBc positive* (but HBsAg negative)	
1. LL/BL	4/6 (66.66%)
2. TT/BT	—
3. Total	4/6 (66.66%)

LL/BL — Lepromatous leprosy.

TT/BT — Tuberculoid leprosy.

*Two sera of this group could not be tested.

for tuberculoid sera. Out of 11 leprosy sera positive for HBsAg, 7 (63.6%) were positive for anti-HBs antibodies also. A total number of 4000 sera from normal persons of the same area were also screened for HBsAg. Out of these sera, 295 were tested for anti-HBs. The per cent positivity of HBsAg and anti-HBs in the normal sera was found to be 3.6% and 21.69%, respectively.

Anti-HBc (IgM) antibodies were detected in 8 out of 134 (6%) lepromatous sera. All these 8 sera belonged to LL patients, and were negative for HBsAg as well as anti-HBs antibodies. In addition, these sera showed normal values of aminotransferases (SGOT and SGPT). None of the tuberculoid sera was positive for IgM anti-HBc antibodies.

To evaluate the status of HBV replication in leprosy patients, the sera positive for HBsAg or anti-HBc antibodies were analysed for HBV-specific DNA polymerase (DNA-P) activity. DNA-P activity was found in 22.22% of HBsAg positive sera and 66.66% of IgM anti-HBC positive sera (Table 2). All the sera positive for DNA-P activity belonged to the LL category.

DISCUSSION

An analysis of sera of lepromatous and tuberculoid leprosy patients for the presence of four HBV markers showed that the per cent positivity of lepromatous sera for HBsAg was higher than that of tuberculoid sera and sera of normal healthy controls. However, the difference in the positivity of the three groups was not statistically significant. Similarly, the difference in the positivity of these three groups of sera for anti-HBs antibodies was also not statistically significant. These observations indicate that the prevalence of HBsAg and anti-HBs antibodies in leprosy patients is comparable to that of the normal population. These data are in agreement to results of various other studies reported earlier for HBsAg (Salzano and Blumberg, 1970; Sher *et al*, 1977; Chakrabarty *et al*, 1979; Papaioannou *et al*, 1986), and anti-HBs antibodies (Ree and Talonu 1981; Serjeantson and Woodfield 1978; Thyagarajan *et al*, 1978).

Anti-HBc IgM was detected in 6% cases of lepromatous group. This marker is an indicator of acute HBV-infection (Lemon *et al*, 1981) but may be present in chronic HBV-infection also (Lindsay *et al*, 1986). Anti-HBc IgM normally appears within two weeks of the onset of disease and may persist upto two years (Fig. 1). Therefore, the presence of IgM anti-HBc in absence of signs and symptoms of the disease indicate a recent past infection where anti-HBc IgM formed during acute phase persists even after

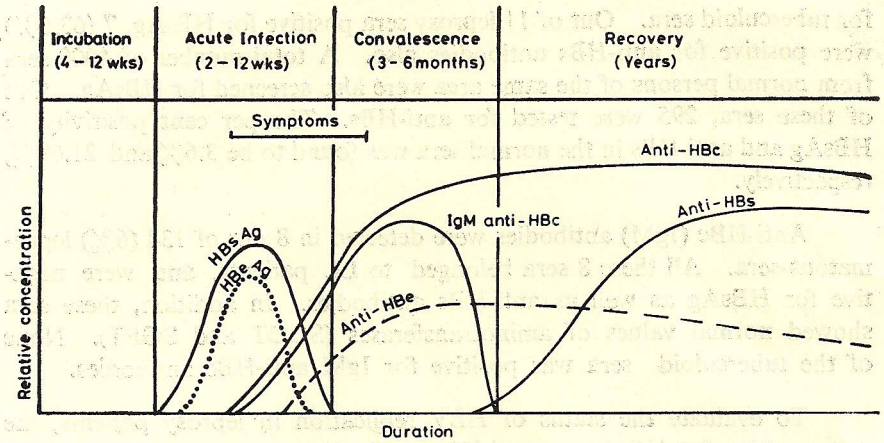

Fig. 1. Showing a generalised pattern of the appearance of different HBV markers in the blood of a typical case of acute viral hepatitis B. (HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis 'e' antigen; Anti-HBe: Antibody to HBeAg; IgM anti-HBc: IgM antibody to hepatitis core antigen; Anti-HBc: IgG antibody to hepatitis core antigen; Anti-HBs: Antibody to HBsAg).

recovery. In the present study, we found that all IgM anti-HBc positive patients had normal levels of serum transaminases (SGOT and SGPT) and were negative for HBsAg. This implies that IgM anti-HBc in these patients is the persistent antibody formed during HBV infection and is still present even after recovery from the disease.

Presence of HBV-associated DNA polymerase (a marker of ongoing HBV replication) in 66.6% of IgM-anti-HBc positive cases indicates that HBV-replication in these patients is still continued but possibly is in the declining phase, whereas HBsAg has already become undetectable. Since anti-HBs usually appears in the convalescent phase i.e., after disappearance of IgM anti-HBc and other markers (Fig. 1), the absence of anti HBs in the present series of IgM anti-HBc positive cases points towards a normal phenomenon where anti-HBs possibly might appear in these patients after the disappearance of IgM anti-HBc. The analysis of HBsAg positive cases for DNA-polymerase showed its presence in 2 out of 9 cases (22.2%). The low HBV replication in leprosy patients as compared to that in healthy HBV-carriers (44%; unpublished data) from the same region, may be explained by the fact that the number of samples analysed is very small. A large number of samples from leprosy patients is needed to be analysed before drawing a conclusion.

The coexistence of HBsAg and anti-HBs in the same sera may be explained by the difference of subtypes of HBsAg and anti-HBs as reported earlier (Hoofnagle and Alter, 1984).

It may be concluded from this study that leprosy patients do not reveal high susceptibility to HBV-infection. The HBV-carrier rate in these patients is nearly the same as in the general population. Although there is low HBV-replication in HBsAg carriers from leprosy patients group, this cannot be the real incidence as the number of samples analysed is very small. The pattern of acute HBV-infection in leprosy patients therefore follows the typical pattern of acute infection in the normal population.

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