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Hepatitis B Virus (HBV) Serum Markers in Greek Leprosy Patients[1]

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The association between hepatitis B surface antigen (HBsAg) and lepromatous leprosy (LL), first reported by Blumberg, *et al.* [4], has been the subject of several controversial investigations. While in some surveys elevated rates of HBsAg were found in LL patients [3, 7, 9, 14, 16], in others the prevalence of HBs antigenemia in leprosy cases was found to be in the "normal" range [11, 17, 20, 21]. From these studies it was concluded that when the prevalence of HBsAg exceeds 1% in the healthy population and the survey sample consists mainly of hospital patients, an association between LL and HBsAg is usually observed [1, 8]. On the other hand, when the prevalence of HBsAg is less than 1% in the general population, high rates of HBsAg are not found in the leprosy cases [17, 21, 22].

Investigators who have found an association between LL and HBsAg favor the hypothesis that the immune defect which predisposes to LL is also responsible for the increased susceptibility to the persistence of HBsAg [7, 16]. Alternatively, immune response genes associated with leprosy are postulated to be correlated with a gene for persistent carriage of the hepatitis B virus [4, 19]. Some studies, however, did not control for the fact that lepromatous patients are more likely to be hospitalized for long periods during which the opportunity for infection is greater. Thus, the argument as to whether the prevalence of HBs antigenemia is indeed higher among lepromatous patients, as a result of some defect, is still unresolved [5].

The purpose of this study was to investigate the degree of exposure of Greek leprosy patients to hepatitis B virus (HBV) and the ability of the exposed leprosy patients to clear the HBV from the circulation. We, therefore, studied the presence of HBsAg, antibody to the hepatitis B surface antigen (anti-HBs), and antibody to the hepatitis B core antigen (anti-HBc) in the sera of patients with the two polar types of leprosy and in the sera of hospital controls. We analyzed the data for two distinct serological patterns: a) effective exposure, characterized by the presence of HBsAg and/or anti-HBs and/or anti-HBc and b) active infection, characterized by the presence of HBsAg.

MATERIALS AND METHODS

The group studied consisted of 217 leprosy cases, 135 of whom had polar lepromatous leprosy and 82 polar tuberculoid leprosy. Cases of borderline and indeterminate forms of leprosy were excluded from the study. The leprosy cases were hospitalized or followed up as outpatients at the Center for Hansen's Disease, Hospital for Infectious Diseases, Athens, Greece. The control group consisted of 382 patients of low socioeconomic class hospitalized in nearby hospitals for diseases other than leprosy. All subjects were of Caucasian origin.

Serum samples were collected from all subjects within a six-month period and were kept at −20°C. HBsAg, anti-HBs, and anti-HBc were detected in the sera of all of the patients and the controls by radioimmunoassay (RIA) (Austria II, Ausab; Corab-Abbott Laboratories). The tests were per-

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TABLE 1. *Distribution of leprosy cases of the two polar types (LL and TT) and of hospital controls according to HBV serum markers.*

Serum markers	LL		TT		Controls	
	Total	%	Total	%	Total	%
Active infection[a]	9	6.7	7	8.5	22	5.8
Anti-HBc + anti-HBs[a]	86	63.7	47	57.3	135	35.3
Only anti-HBc[a]	18	13.3	12	14.7	48	12.6
Only anti-HBs[a]	3	2.2	5	6.1	22	5.8
Absence of markers	19	14.1	11	13.4	155	40.5
Total	135	100	82	100	382	100

[a] The sum of these four categories refers to individuals with effective exposure.

formed blind and were completed within two months of collection of the sera.

RESULTS

Two distinct serological patterns were operationally utilized: a) effective exposure, which is characterized by the presence in the serum of HBsAg and/or anti-HBs and/or anti-HBc, and b) active infection, which is characterized by the presence in the serum of HBsAg with or without the presence of antibodies.

Table 1 shows the distribution of the leprosy cases and of hospital controls according to HBV serum markers. The prevalence of HBsAg was 6.7% among LL cases, 8.5% among TT cases, and 5.8% among hospital controls.

Table 2 shows the distribution of 82 TT leprosy cases, 135 LL cases, and 382 hospital controls according to sex, age (large groups), and serological HBV patterns. Statistical analysis with the χ statistic (not χ^2) was done after stratification by age and sex with the procedure introduced by Mantel and Haenszel ([13]). The prevalence of effective exposure was compared between the two groups of leprosy cases and the hospital controls. It was found that TT as well as LL cases had a higher prevalence of effective exposure in comparison to the controls ($\chi = +4.45$, $p < 10^{-5}$ and $\chi = +5.12$, $p < 10^{-6}$, respectively). The difference remained statistically significant when all leprosy cases were combined ($\chi = +5.21$, $p < 10^{-6}$). No statistically significant difference was found between the two polar leprosy groups ($\chi = -0.19$, $p > 0.30$) and no significant difference was found between bacteriologically positive and bacteriologically negative LL cases ($\chi = -0.17$, $p > 0.30$, data not shown).

The prevalences of active infection among the effectively exposed subjects of the two different groups of leprosy cases and of hospital controls were compared using as before age and sex stratification and the Mantel-Haenszel procedure ([13]). It was found that no statistically significant difference existed between TT cases and controls ($\chi = +0.49$, $p > 0.30$); LL cases and controls ($\chi = -0.39$, $p > 0.30$); the two polar leprosy groups combined and controls ($\chi = -0.11$, $p > 0.30$); the two polar leprosy groups ($\chi = -0.55$, $p > 0.30$); and LL cases bacterio-

TABLE 2. *Distribution of 82 polar tuberculoid leprosy cases (TT), 135 polar lepromatous leprosy cases (LL) and 382 hospital controls (C), according to sex, age (large groups), and serological patterns for hepatitis B.*

Serological patterns	Males						Females					
	<49 yrs			≥50 yrs			<49 yrs			≥50 yrs		
	TT	LL	C	TT	LL	C	TT	LL	C	TT	LL	C
Active infection[a]	0	1	4	3	4	12	1	1	6	3	3	0
Effectively exposed[b]	10	11	20	27	59	126	11	11	57	23	35	24
Total tested	13	15	34	31	70	190	12	11	120	26	39	38

[a] Presence of HBsAg.
[b] Presence of HBsAg and/or anti-HBs and/or anti-HBc.

TABLE 3. *Distribution of 382 hospital controls according to age and serological patterns of hepatitis B.*

Serological patterns[a]	Years of age				Total
	<39	40–49	50–59	≥60	
Active infection	4	6	5	7	22
Effectively exposed	41	36	42	108	227
Total tested	89	65	74	154	382
Active infection as % of effectively exposed	10%	17%	12%	7%	10%
Effective exposure as % of total	46%	55%	57%	70%	59%

[a] See footnotes for Table 2.

logically positive and those negative ($\chi = +0.79$, $p > 0.30$, data not shown).

The distribution of the hospital controls and the leprosy cases according to age, polar type, and serological patterns of hepatitis B is shown in Tables 3, 4, and 5.

The statistical evaluation of a possible trend of the HBV markers with age, after appropriate sex adjustment, was done by the Mantel-extension procedure ([12]). The results are as follows: a) In the control group, there was a statistically significant increase in the prevalence of effective exposure with age ($\chi = +2.56$, $p < 0.05$). In the two polar leprosy groups, a similar increase existed but it was not statistically significant ($\chi = +0.93$, $p > 0.30$ and $\chi = +1.02$, $p > 0.25$). b) In the control group, there was a statistically significant decrease in the prevalence of active infection among exposed individuals with age ($\chi = -2.19$, $p < 0.05$). On the contrary, among leprosy cases of either type a significant decrease was not found ($\chi = +0.60$, $p > 0.30$ and $\chi = -0.10$, $p > 0.30$).

DISCUSSION

The lack of agreement between various studies, as far as the prevalence of HBsAg among leprosy patients is concerned, may be due to a variety of factors, such as the geographical area, the sample of patients, the sample of control subjects, and the methodology for testing serological parameters and for analyzing the results. The factors which have been held responsible for increased antigenemia (where it was found) are, on the one hand, the probable immunological defect in leprosy subjects ([7, 16, 18]) and, on the other hand, increased risk of exposure to the virus ([10, 19]). However, it has not been proved that there is really a nonspecific immunological defect in leprosy subjects, and there are no studies which establish the proportion of leprosy patients effectively exposed to HBV infection after investigating all of the serological parameters. Thus, no indisputable interpretation can be given to the "increased" prevalence

TABLE 4. *Distribution of 82 polar tuberculoid leprosy cases (TT) according to age and serological patterns.*

Serological patterns[a]	Years of age				Total
	<39	40–49	50–59	≥60	
Active infection	1	0	2	4	7
Effectively exposed	7	14	18	32	71
Total tested	9	16	22	35	82
Active infection as % of effectively exposed	14%	0%	11%	13%	10%
Effective exposure as % of total	78%	88%	82%	91%	87%

[a] See footnotes for Table 2.

TABLE 5. *Distribution of 135 polar lepromatous leprosy cases (LL) according to age and serological patterns of hepatitis B.*

Serological patterns[a]	Years of age				Total
	<39	40–49	50–59	≥60	
Active infection	0	2	3	4	9
Effectively exposed	4	18	38	56	116
Total tested	7	19	45	64	135
Active infection as % of effectively exposed	0%	11%	8%	7%	8%
Effective exposure as % of total	57%	95%	84%	88%	86%

[a] See footnotes for Table 2.

of HBsAg which has been found in various studies. Hence, it is of primary importance to establish the population of subjects exposed to HBV and to investigate the prevalence of antigenemia within this population. It should be noted that the degree of exposure to the virus in nonleprosy individuals is probably different, and that exposed leprosy patients should be compared with a population on nonleprosy cases exposed to HBV.

In the present study, by using a sensitive radioimmunoassay to search for three serological markers of HBV (HBsAg, anti-HBc, anti-HBs), we were able to investigate two parameters: a) the proportion of subjects effectively exposed to HBV, and b) the proportion of subjects with active HBV infection. Exposed subjects were considered to be those in whom one or more of the serological markers of HBV were found, while subjects with active infection were considered to be those in whom the presence of HBsAg in the serum was established with or without the other two serological markers.

The investigation of these serological parameters established that, in our material, the prevalence of effectively exposed subjects in patients with lepromatous leprosy, in patients with tuberculoid leprosy, and in hospital controls was approximately 86%, 87%, and 59%, respectively. Statistical analysis revealed that the leprosy patients of both polar types had a significantly greater prevalence of effective exposure to HBV than control patients. On the contrary, no statistically significant or biologically important difference was found concerning the degree of exposure to HBV between the two polar types of the disease and between patients with lepromatous leprosy with a positive or negative bacterial index (BI).

These comparisons show that the leprosy patients studied, who were a representative sample of all Greek leprosy patients treated at the Center for Hansen's Disease in Athens, have an increased exposure to HBV when compared to hospital controls. The absence of any significant difference in HBV exposure between the two polar types of the disease and between leprosy patients with a positive or negative BI implies that the increased prevalence is due not to biological parameters but to the greater opportunity for infection. Other workers([7, 10]) also believe that leprosy patients will manifest a greater probability of active exposure to HBV because of their protracted stay in a hospital environment.

The basic purpose of this study was to find the proportion of HBsAg carriers among leprosy cases effectively exposed to HBV, and to compare the findings with those from a corresponding sample of effectively exposed nonleprosy cases in order to draw conclusions concerning the biological factors which determine the course of HBV infection in leprosy patients.

The results of our study indicate that the proportion of subjects with active infection among those effectively exposed to HBV does not differ among groups of patients with tuberculoid leprosy, lepromatous leprosy with a positive or negative BI, or hospital controls. These findings do not implicate special biological factors responsible for the development of HBV antigenemia among leprosy patients, although these factors may play a role in other situations or

under different conditions ([2]). It is highly likely that the establishment of HBs antigenemia is of multifactorial origin ([23]), but our findings indicate that exogenous environmental factors are the critical determinant of HBs antigenemia among leprosy cases in Greece. Our results are in accordance with several previous studies which did not find an increased prevalence of antigenemia in leprosy patients ([15, 17, 20, 21]) and challenge the results of those which found increased HBV antigenemia in leprosy patients (particularly in those with the lepromatous type of the disease) because of alleged disturbances of the nonspecific cell-mediated immunity among these patients ([7, 9]). The disagreement may be due to differences in the samples of leprosy cases studied, differences in the control groups used, or to methodological differences in both the tracing of serological parameters and the evaluation of results. Our leprosy cases of both types were under continuous medical surveillance and lived under better conditions than many cases used in previous studies, which may have a beneficial effect on the immunological status. Nevertheless, the lack of a correlation between the BI and active HBV infection does not support the hypothesis that reduced nonspecific cellular immunity is responsible for the persistent antigenemia ([15, 20]).

In our study the only indication of an inability of leprosy cases to clear HBsAg from the circulation was the lack of a downward trend with age in the prevalence of active HBV infection among patients effectively exposed; whereas among controls, such a trend was both obvious and statistically significant. This indication is, however, rather weak and the main conclusion of our study is that leprosy patients of any polar type do not have an inherently increased susceptibility to the infection by hepatitis B virus or to persistent HBs antigenemia following an infection by this virus.

SUMMARY

The prevalence of HBsAg, anti-HBs, and anti-HBc in the sera of 217 patients with the two polar types of leprosy and 382 hospital controls was studied in order to investigate the degree of exposure of Greek leprosy patients to HBV and the ability of these patients to clear HBV from the blood. Two distinct serological patterns were analyzed: a) effective exposure, characterized by the presence of one or more of the three serological markers, and b) active infection, characterized by the presence of HBsAg.

From the statistical analysis it was found that TT as well as LL cases had a higher prevalence of effective exposure in comparison to controls ($p < 10^{-5}$ and $p < 10^{-6}$). No significant difference was found between the two polar leprosy types ($p > 0.30$) or between bacteriologically positive and negative LL cases ($p > 0.30$).

As far as the prevalence of active infection is concerned among the effectively exposed subjects of all groups, no significant difference existed between TT cases and controls, LL cases and controls, the two polar leprosy cases combined and controls, the two polar leprosy groups, and LL cases positive and negative for *Mycobacterium leprae* (p for all comparisons >0.30).

It is concluded that leprosy cases are at a high risk of HBV infection, but the prevalence of active infection among those effectively exposed does not differ between leprosy cases and hospital controls.

RESUMEN

Para poder investigar el grado de exposición de los pacientes Griegos con lepra al virus de la hepatitis B (HBV) y su capacidad para eliminarlo de circulación, se estudió la prevalencia del antígeno HBsAg y de los anticuerpos anti-HBs y anti-HBc en el suero de 217 pacientes con alguno de los dos tipos polares de la lepra y en el suero de 382 controles de hospital. Se analizaron 2 patrones serológicos distintos: a) la exposición efectiva caracterizada por la presencia de uno o más de los 3 marcadores serológicos y b) la infección activa caracterizada por la presencia de HBsAg.

Se encontró que tanto los pacientes TT como los LL tuvieron una mayor prevalencia de exposición efectiva que los individuos del grupo control ($p < 10^{-5}$ y $p < 10^{-6}$). No se encontró una diferencia significativa entre los dos tipos polares de lepra ($p > 0.3$) ni entre los casos LL bacteriológicamente positivos y los negativos ($p > 0.3$).

En cuanto a la prevalencia de la infección activa entre los sujetos efectivamente expuestos, no se encontraron diferencias significativas entre los casos TT y los controles, entre los casos LL y los controles, entre los casos LL y TT, ni entre los casos LL bacteriológicamente positivos y los negativos (en todos los casos los valores de p fueron >0.3).

Se concluye que los pacientes con lepra tienen un alto riesgo de infección por el HBV pero que la prevalencia de infección activa en los individuos efectivamente expuestos es igual a la encontrada en los controles de hospital.

RÉSUMÉ

En vue d' explorer le degré d'exposition de malades grecs atteints de lèpre à HBV et la capacité de ces malades à éliminer HBV de leur sang, on a étudié la prévalence de HBsAg, d'anti-HBs et d'anti-HBc dans le sérum de 217 malades atteints de l'une ou l'autre des formes polaires de lèpre, et de 382 témoins choisis parmi les malades hospitalisés.

Deux profils sérologiques distincts ont été analysés: a) une exposition réelle caractérisée par la présence d'un ou plusieurs des trois marqueurs sérologiques; b) une infection active, caractérisée par la présence de HBsAg. L'analyse statistique a permis de montrer que les cas TT aussi bien que les cas LL présentaient une fréquence plus élevée d'exposition réelle, par comparaison aux témoins ($p < 10^{-5}$ et $p < 10^{-6}$). Aucune différence significative n'a été trouvée en fonction des deux types polaires de la lèpre ($p > 0.30$), ou du caractère bactériologique positif ou négatif des cas LL ($p > 0.30$).

En ce qui concerne la prévalence d'infection réelle parmi les sujets réellement exposés dans chacun des groupes, aucune différence significative n'a été mise en évidence entre les cas TT et les témoins, les cas LL et les témoins, les cas des deux types polaires de la lèpre combinés, d'une part, et les témoins d'autre part, les deux types polaires de la lèpre, et les cas LL respectivement positifs ou négatifs pour *Mycobacterium leprae* (la probabilité pour toutes ces comparaisons était supérieure à 0.30).

On en conclut que les cas de lèpre présentent un risque élevé d'infection HBV mais que la prévalence d'une infection active parmi ceux qui ont été réellement exposés ne diffère pas entre les cas de lèpre et les témoins hospitaliers.

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