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Changes in serum iron levels due to infection with hepatitis B virus

(iron/hepatitis B/carrier/bacterial infection)

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ABSTRACT **We found in two previous studies (Down syndrome patients and end-stage kidney patients receiving renal dialysis) that total serum iron is higher on average in carriers of the hepatitis B virus than in those who are not. The elevation of the serum iron is independent of elevations of serum L-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2) (SGPT), an indicator of liver cell damage. We have followed for 10 yr a large number of patients with end-stage renal disease receiving renal dialysis. In this paper we describe studies of serum iron and SGPT levels in patients (*i*) 1 mo before infection, (*ii*) after infection but within the month of infection, and (*iii*) 6–12 mo after infection. Comparisons of serum iron levels were made between those infected who retained the virus (carriers) and those who rejected the infection (transients). There were no differences between these groups before infection. Serum iron remained high in the carrier group and dropped in the transients. However, not all of the carriers retained high levels, although this was the case in general. Individual changes in the pre- and postconversion period were then considered. All carriers who had a preconversion decline in iron had an increase after infection, whereas this occurred in only some of the transients. Those carriers who had a decline after infection had raised levels before infection, and the decline was generally less than the increase. Consideration of the SGPT and the iron levels together led to the same conclusion as the previous studies, that elevation of iron may be independent of rise in SGPT. Several hypotheses were derived from these findings. Individuals who are carriers in general have higher iron levels and, therefore, are more likely to become infected with bacteria; this may contribute to increased morbidity and mortality. From experimental evidence, iron is required for the growth of tumor cells. Carriers with elevated iron levels may be more likely to develop detectable cancer of the liver than those who do not.**

Serum iron appears to play an important role in the body's defense against many bacterial infections (1). The response of the body after infection is to decrease iron absorption through the intestine, which is normally the major source of body iron. This has the effect of decreasing the iron available to the infecting organism and, thereby, decreasing its probability of survival. Iron is also required for the growth of all living cells, including tumor cells. Fernandez-Pol (2) has shown that a plant substance, picolinic acid, inhibits the growth of tumor cells in tissue culture as a consequence of the chelation of iron (but not zinc), thereby depriving the cells of a necessary growth substance. Serum iron is known to be elevated in hepatitis, presumably as a result of the disruption of liver cells and the addition of their iron content to that of the serum.

Until recently there was not much information on iron levels in carriers of hepatitis B virus (HBV). We became interested in understanding more about this phenomenon because of the accumulating evidence to support the hypothesis that persistent infection with HBV is required for the development of most cases of primary hepatocellular carcinoma (PHC) (3).

Previously, we studied two groups of patients with high frequencies of HBV carriers. In male Down syndrome patients, we found carriers to have significantly higher levels of serum iron and higher levels of percent serum iron saturation than patients who were not carriers (4). The iron levels were not correlated with serum glutamic-pyruvic transaminase (SGPT; serum L-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2) levels (a liver enzyme whose presence indicates liver damage). A similar study was done in patients with end-stage renal disease treated at a renal dialysis unit in Philadelphia, PA (5). Again, the serum iron levels and percentage iron bound were higher in the carriers than noncarriers. There was a correlation with SGPT and also an independent correlation with the presence of HBV. Both of these studies are consistent with the explanation that the increase in iron was not only the result of liver cell breakdown but of the presence of the HBV itself.

These observations do not distinguish between the alternatives that the serum iron elevation is a consequence of hepatitis infection or that the carrier state is a consequence of a raised serum iron level. This paper reports a longitudinal study of patients who become infected with HBV during the course of chronic hemodialysis treatment and demonstrates that serum iron levels in carriers are not raised prior to infection.

METHODS

Patient Population. For the past 9 years, we have been following patients with end-stage renal disease receiving renal dialysis at a commercial hemodialysis unit in the Delaware Valley (6). Serum specimens are collected from each patient two times per month starting from the time of their admission to the clinic and are tested for the presence of HBV surface antigen (HBsAg) and other measures of response to HBV infection. From our serial samples, the time of seroconversion can be determined, and the patient can be classified as either a carrier [persistently infected, HBsAg(+), for >6 mo] or a transient [transiently infected, HBsAg(+), for <6 mo]. We have found that, in this clinic, the probability of remaining HBsAg(+) indefinitely after being positive for at least 6 mo exceeds 90% (6).

There were 34 transients and 33 carriers. Three samples over time were tested from each of these patients: (*i*) 1 mo before seroconversion to HBsAg(+); (*ii*) first drawing after seroconversion to HBsAg(+); and (*iii*) 6–12 mo after seroconversion.

Assays. Iron was detected spectrophotometrically on all the samples at about the same time with Hyland Ferro-chek II kits (Traveral, Costa Mesa, CA). These tests were done months or years after collection by using serum stored at −20°C. HBsAg was determined by the Ausria II method (Abbott). SGPT was

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Abbreviations: SGPT, serum glutamic-pyruvic transaminase (L-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2); HBV, hepatitis B virus; HBsAg, HBV surface antigen; PHC, primary hepatocellular carcinoma.

determined within a few days of collection of the sera by a modification of the method of Henry *et al.* (7).

RESULTS

Serum iron was not higher before infection in the carriers-to-be than it was in the patients destined to be transients (Table 1). The mean iron was elevated immediately after infection in both groups. It remained high in the carrier group but dropped to preinfection levels in the transient group. In general, iron elevation appeared to follow the infection rather than precede it.

The change in iron levels from the initial sample to the final sample was significantly different in carriers and transients, when considered as individuals. For transients, the mean change was approximately 1 mg/dl with the quartiles at −19 mg/dl and 25 mg/dl. For carriers, the mean change was approximately 24 mg/dl with the quartiles at 0 mg/dl and 53 mg/dl. The standard error of the mean change in iron levels was approximately 7.3 in carriers and 8.8 in transients. Thus, the change from initial sample to final sample in carriers was significantly greater than in transients ($P < 0.04$, one-tailed test), as would be predicted from earlier studies.

In addition, the pattern of changes in iron levels was considerably different. In approximately 35–40% of both carriers and transients, levels of serum iron decreased from the first to the second sample (Table 2, preseroconversion change). Between the second and third samples (postseroconversion change), a similar fraction of carriers had decreasing iron, whereas the same fraction of transients had an increasing iron level (Table 2, postseroconversion change). Hence, the propensity to remain a carrier is not controlled by an elevated serum iron. However, the correlation between increasing iron levels and the carrier status is quite clear.

These pre- and postseroconversion iron level changes were considered in combination (Table 2, combination). All 12 carriers who had a preseroconversion decline in iron had an increase in iron after seroconversion, whereas 7 of 14 transients declined in both time periods ($P < 0.01$, one-tailed test). Similarly, all 12 carriers who had a decline in iron after infection had an increase before infection, whereas only 15 of 22 transients had the same pattern ($P = 0.03$, one-tailed test). That is, there were no carriers who had declining iron in both time periods. From the data on individuals, this represents the only consistent observation that can be made with the iron data alone.

By using SGPT, however, additional insight into the interaction between liver cell breakdown, iron levels, and HBV is possible. In Table 3, the postseroconversion (sample 2 to sample 3) SGPT and serum iron levels are considered in combination. Note that most transients had decreasing iron and SGPT levels, and most carriers had increasing levels of iron and SGPT. A statistical analysis of this table using a logarithmic-linear model (8) shows that an excellent fit of the data is obtained by including interactions between HBV and iron and between HBV and SGPT. The residual χ^2 is only 0.60 for this model. SGPT and iron do not interact in this model, which is consistent with previous observations that the association of increasing serum iron with the carrier state does not directly depend on liver cell breakdown. This analysis, which is based on patterns of change over time, reaches the same conclusions as the previous work, which was based on the magnitudes of the serum iron and SGPT levels at one point in time.

Table 1. Mean iron levels in carriers and transients before (sample 1), immediately after (sample 2), and 6–12 mo after (sample 3) seroconversion

	Mean iron levels, μg/dl		
HBV status	Sample 1	Sample 2	Sample 3
Carrier	96.7	115.3	119.6
Transient	100.7	115.8	101.4

For carriers, the mean iron levels before seroconversion were significantly less than the other two values ($P < 0.05$).

Table 2. Serum iron level changes over time

Iron level changes			
Postseroconversion	Preseroconversion	Carriers	Transients
—	Increased iron	21	20
—	Decreased iron	12	14
Increased iron	—	21	12
Decreased iron	—	12	22
	Combination		
Increased iron	Increased iron	9	5
Increased iron	Decreased iron	12	7
Decreased iron	Increased iron	12	15
Decreased iron	Decreased iron	0	7

Preseroconversion refers to the change between the first and second samples; postseroconversion refers to the change between the second and third samples.

DISCUSSION

There are several biological hypotheses which arise from these findings: (*i*) The increase in serum iron in carriers could contribute to increased hemoglobin metabolism. This hypothesis can be tested by studies of iron and erythrocyte metabolism in relation to HBV response. Such a study ought to include a number of other factors, such as ferritin, because both the carrier and transient populations appear to be heterogeneous in their changes in serum iron levels.

(*ii*) A variety of studies recently reviewed have shown that increased iron levels and particularly increased percentage of iron saturation increase the probability of infection with a variety of bacteria. This generates the hypothesis that carriers of the hepatitis virus are more likely to have bacterial infections and that such infections would be more severe than in transiently infected persons. Our laboratory has been studying the effects of HBV infection on fertility and mortality. In general, carriers have decreased fertility and increased mortality. If this hypothesis concerning the bacteria is true, it would provide a partial explanation of the demographic and epidemiologic observations that we have made.

(*iii*) We recently have summarized the data that provides strong support for the hypothesis that persistent infection with

Table 3. Postseroconversion change in SGPT and serum iron levels*

SGPT	Iron	Carrier†	Transient†
Increase	Increase	19 (58)	7 (21)
	Decrease	7 (21)	4 (12)
Decrease	Increase	2 (6)	5 (15)
	Decrease	5 (15)	18 (55)
		33 (100)	34 (100)

* From sample 2 to sample 3 (from immediately after conversion to 6–12 mo later).

† The number of individuals precedes the percentage in parentheses.

HBV is necessary for the development of PHC. According to this model, nearly all patients with PHC have been persistently infected with HBV, but only a fraction of the carriers will proceed through chronic liver disease on the unfortunate path to PHC (3). Are the serum iron levels and iron metabolism related to this? In studies of PHC tumor tissues, it has been found that the cancer cells have much less stainable iron storage than nonmalignant cells (9). By using fluorescent and other indicators for HBsAg and the HBV core antigen, it has been shown that the virus is present in the liver cells of patients with PHC; but the transformed cells usually lack or have small amounts of virus. Fernandez-Pol has found that removal of iron decreases the growth of experimental liver tumors in tissue cultures. Therefore, the survival of transformed liver cells may depend upon surrounding cells providing the iron needed for growth. Hence, we conjecture that carriers with increased iron levels and levels of factors related to iron would be at greater risk of developing clinically evident liver cancer than carriers with "normal" iron levels and metabolisms.

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