

Frequency of pregnant women with HB_sAg in a Brazilian community¹

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SUMMARY

The work reported here points up the real benefits provided by neonatal immunoprophylaxis of newborns delivered by mothers who are seropositive for the hepatitis B virus surface antigen HB_sAg and underscores the need to properly identify such mothers in Brazil so that immunoprophylaxis can be undertaken. To help determine levels of hepatitis B virus (HBV) infection and seropositivity for various HBV markers among pregnant women in Southeast Brazil, investigators studied 7992 pregnant women delivering at the Clinical Hospital of the University of São Paulo's Ribeirão Preto School of Medicine in Ribeirão Preto, Brazil. Seroreactivity for HB_sAg was determined first by serologic screening with an enzyme-linked immunosorbent assay (ELISA) procedure in which the sera were incubated for 2 hours and then by confirmation with another ELISA in which the sera were incubated for 18 hours. Subsequently, tests for anti-HB_sAg, HB_eAg, anti-HB_eAg, and anti-HB_cAg markers were conducted using confirmed positive samples. Initial screening found 84 of the 7992 samples (1.05%, 95% CI: 0.84-1.30) to be positive for HB_sAg; however, this HB_sAg positivity was confirmed in only 76 (0.95%, 95% CI: 0.75-1.19). The positivity rate was significantly higher among subjects whose pregnancies terminated in miscarriage (1.84%) than among those with live births (0.83%) (χ^2 , Yates correction = 7.6; $P = 0.005$). Anamnesis was able to identify HBV risk factors in only 27.6% of the confirmed HB_sAg-positive subjects or close household contacts. However, 21.3% (95% CI: 1.04-30.56) of the confirmed HB_sAg-positive subjects were found positive for HB_eAg, indicating a high risk of vertical transmission of the virus. These results demonstrate a need to conduct specific serologic research at term, in order to provide effective neonatal immunoprophylactic benefits.

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It is known that hepatitis caused by B virus (HBV) occurs with greater frequency among young adults whose occupations, lifestyles, or behaviors (particularly illicit intravenous drug use and unprotected homosexual or heterosexual activities) place them at increased risk of contracting the infection. Among other things, HBV transmission through heterosexual relations accounts for some 25% of all hepatitis B cases, a higher percentage than that transmitted through homosexual relations (1-3). More specifi-

cally, in the case of Brazil the high level of heterosexual transmission has resulted from an increase in the number of HBV-infected women of reproductive age (4).

One of the most important routes of HBV transmission and sources of HBV perpetuation in the community, of concern to both pediatricians and obstetricians, is mother-child transmission (5, 6). Transcending specific epidemiology, such perinatal HBV transmission can occur as a result of either acute or chronic maternal infec-

tion. Other researchers (7) have found the risk of vertical HBV transmission to be 71% when acute maternal infection occurs at the end of pregnancy versus 3% when it occurs at the onset of pregnancy. It has also been shown that the fetuses or newborns of women who are chronic HB_sAg and HB_eAg carriers are more susceptible to vertical transmission (80-90% risk), a finding that highlights the importance of assessing all the serologic markers of HBV infection (8, 9).

Approximately 6% of the cases of perinatal HBV transmission occur during pregnancy (10), while the remainder occur during or shortly after birth. This high prevalence of relatively late transmission permits immunoprophylaxis initiated after pregnancy to serve as an effective measure for reducing neonatal infection—as well as preventing acute and chronic complications (11, 12).

According to data released by the World Health Organization's Consulting Group on Hepatitis (13), active immunoprophylaxis is indicated for all newborn babies in communities where the prevalence of HBV is intermediate or high (over 2%). In the meantime, until universal prophylaxis is instituted in communities where HBV prevalences are high and also where the prevalences are below 2%, the viable alternative is detection of pregnant women who are HB_sAg carriers and provision of prophylaxis, a procedure that not only determines the seroprevalence of infection but also permits development of regional strategies for addressing the problem and selective application of neonatal HBV immunoprophylaxis (11). This measure is directed at reducing and controlling vertical HBV transmission (14).

Although identifying pregnant women at risk of hepatitis B based on their clinical histories used to be controversial, there is no longer any doubt that serologic screening for HB_sAg is clearly superior to anamnesis for this purpose (10, 15-19), or that it shows a cost-benefit ratio that supports its use (14, 20-22).

In Brazil, HBV infection is most prevalent in the Amazon region, reach-

ing levels as high as 12% in some communities (23). The prevalence is lower in the southeastern area, ranging from 0.1% to 4.0% (24). However, information about the HBV prevalence among pregnant or postpartum women in Brazil is scanty (25), which is one of the prime reasons for the study reported here.

The aims of this study were as follows: (1) to determine the rate of HB_sAg seropositivity among women whose pregnancies were resolved at the Clinical Hospital of the University of São Paulo's Ribeirão Preto School of Medicine in Ribeirão Preto, Brazil; (2) to assess the contribution of anamnesis to the detection of HBV risk factors among women carrying Hb_sAg; and (3) to confirm the accuracy and significance of a positive test for HB_sAg by conducting tests to verify the presence of this antigen in the serum and to detect other serologic markers of HBV infection.

MATERIALS AND METHODS

The study was conducted at the Clinical Hospital of the Ribeirão Preto School of Medicine from 1 March 1991 through 31 December 1993. During this period the 7992 women whose pregnancies were resolved at the hospital were examined for seroreactivity to HBV surface antigen (HB_sAg).

Detection of HB_sAg seroreactivity was achieved in two steps (screening, followed by confirmatory testing) using an enzyme-linked immunosorbent assay (Auzyne ELISA, Abbott Laboratories). In the first procedure (screening), the serum samples tested were incubated for two hours. To exclude possible false-positives, the samples testing positively were then retested using the same ELISA procedure with a longer incubation period (18 hours). In addition, samples that were repeatedly positive for HB_sAg were tested for other serologic markers of HBV infection using other immunoenzymatic methods: anti-HB_sAg (Ausab EIA, Abbott Laboratories, procedure B), anti-HB_eAg (Corzyme, Abbott Laboratories, procedure B),

HB_eAg and anti-HB_eAg (HB_e-rDNA-EIA, Abbott Laboratories.)

The screening test results were available six to 18 hours after delivery, which made it possible to provide active/passive immunoprophylaxis for the newborns (12). Even when maternal reactivity was not confirmed, the opportunity to complete immunization of the infant was always taken, with additional booster vaccinations being scheduled for 1 and 6 months of age. Cases of confirmed reactivity were evaluated individually, and the women involved were informed about the importance of contraception, use of condoms, and seeking out close contacts (both sexual partners and others) for purposes of vaccination.

Routine evaluation of all new mothers included the gathering of information regarding their professions, habits, sexual behaviors, and illnesses both before and during pregnancy. The following clinical information was obtained from patients identified as HB_sAg carriers:

- Past personal illnesses (hepatitis, chronic renal insufficiency, and other sexually transmitted diseases);
- Problems during the current pregnancy (hepatitis, other sexually transmitted diseases, renal insufficiency, jaundice and/or choluria);
- Professional or behavioral risks (being a health care provider, day care center worker, or sex worker; use of illicit intravenous drugs; alcoholism; imprisonment).

The relationship between seroreactivity for HB_sAg and the way in which pregnancy terminated was tested by means of the chi-square method, assuming a significance level of $\alpha = 5\%$. The results were expressed in 95% confidence intervals (CI).⁵

⁵ Although confidence intervals were calculated to get some idea of the variability of the estimates, it is important to note that the 7992 women studied did not constitute a random sample of the general population of women, so that due caution must be exercised in making inferences about population-based values.

RESULTS

As Table 1 shows, the initial screening test found 84 serum samples from the 7992 study women (1.05%, 95% CI: 0.84-1.30) to be positive. The confirmatory test found 76 of these 84 samples to be positive (0.95% of the total screened, 95% CI: 0.75-1.19). The remaining 8 samples were negative, indicating a false-positive rate of 9.5% (95% CI: 4.2-17.9) for the initial tests employing the shorter incubation period.

The Table 2 data indicate that the prevalence of HB_sAg positivity was higher among women whose pregnancies ended in miscarriage (1.84%, 95% CI: 1.11-2.99) than among those whose pregnancies ended in successful delivery (0.83%, 95% CI: 0.64-1.08). This difference was statistically significant ($\chi^2 = 7.76$; $P = 0.005$).

The routine anamnesis following resolution of pregnancy identified situations and behaviors placing 15 women confirmed positive for HB_sAg and 10 close household contacts of women confirmed positive at risk of HBV infection. Since there was some overlapping (in four cases a woman participating in the study and a close household contact of the same woman were identified as being at risk), the anamnesis identified only 21 (27.6%) of the 76 women confirmed seropositive. Among the risk factors, illicit intravenous drug use was the one most frequently identified among the pregnant women carrying HB_sAg, and prior diagnosis of viral hepatitis was the one most frequently identified among their contacts (Table 3). No cases were detected where the infected women were health professionals, day care center employees, prostitutes, or prisoners.

Table 4 summarizes the results of tests for other serologic markers of HBV infection. For technical reasons, it was not possible to subject all 76 HB_sAg-positive samples to all of the other test procedures. However, the presence of anti-HB_cAg confirmed the presence of HBV infection in 65 (90.3%) of 72 cases found repeatedly positive for HB_sAg. On the other hand,

TABLE 1. HB_sAg seroreactivity among the 7992 study women (who resolved their pregnancies at the Clinical Hospital of the Ribeirão Preto School of Medicine from 1 March 1991 through 31 December 1993), as indicated by the results of two HB_sAg ELISA procedures with incubation periods of 2 and 18 hours.

Test	Positive		Negative		Total	
	No.	%	No.	%	No.	%
HB _s Ag screening	84	1.05 ^a	7908	98.95	7992	100.00
HB _s Ag confirmation	76	0.95 ^b	7916 ^c	99.05	7992	100.00

^a 95% CI: 0.84-1.30

^b 95% CI: 0.75-1.19

^c Only the 84 positive samples received confirmatory testing.

TABLE 2. HB_sAg seroreactivity among study women whose pregnancies terminated in miscarriages, as compared to those whose pregnancies ended in live births.

Result of pregnancy	HB _s Ag positive (confirmed)		HB _s Ag negative		Total	
	No.	%	No.	%	No.	%
Miscarriage	17	1.84 ^a	906	98.16	923	100
Delivery (live birth)	59	0.83 ^b	7010	99.17	7069	100
Total	76	0.95	7916	99.05	7992	100

χ^2 (Yates correction) = 7.76; $P = 0.005$

^a 95% CI = 1.11-2.99

^b 95% CI = 0.64-1.08

the anti-HB_sAg marker was found in only 3 (4.5%) of 67 sera from confirmed HB_sAg-positive cases; and the HB_eAg marker was found in 16 (21.3%) of 75 sera from confirmed HB_sAg-positive cases.

DISCUSSION AND CONCLUSIONS

The medical, health, and economic importance of vertical HBV transmission (the primary form of transmission of this virus during the first decade of life) is undeniable (26). Among other things, it is known that most children infected with HBV early in life become chronic carriers of the infection capable of spreading it to the community (8, 9); and chronic HBV infection is known to cause cirrhosis and hepatocellular carcinoma in adults (27). In a large number of cases, these complications could be prevented by neonatal immunoprophylaxis, a measure that has proved beneficial to both children and their communities.

In the current case, where a positive screening result was obtained in 1.05% and confirmed in 0.95% of the 7992 study women, the screening test made it possible to adopt measures such as active and passive neonatal immunoprophylaxis in the first hours after delivery, a time at which the efficacy of this treatment is more evident than at others (28). A negative confirmatory test could have precluded continuation of the immunization schedule, but instead the opportunity was taken to carry on and complete the newborns' immunization. In this study the confirmatory tests made it possible to effectively identify cases where it was necessary to search for contacts. Contacts of the infected study subjects received counseling on HBV serologic evaluation and anti-HBV vaccination. Sexual partners received additional information about use of condoms during sexual intercourse until the occurrence of postvaccination seroconversion. And both the mothers and their partners were instructed about the need to receive periodic booster vaccinations

TABLE 3. Illnesses, symptoms, behaviors, and experiences indicative of HBV or risk of HBV infection that were identified through anamnesis of the 76 HB_sAg seropositive study women.

Risk factor identified	Patients (n = 15)		Household contacts (n = 10)		Total	
	No.	%	No.	%	No.	%
Illicit IV drug use	6	22.2	4	36.4	10	26.3
Hepatitis	3	11.1	5	45.4	8	21.1
Syphilis	5	18.6	0	...	5	13.2
Jaundice	3	11.1	2	18.2	5	13.2
HIV+	3	11.1	0	...	3	7.9
Alcoholism	3	11.1	0	...	3	7.9
Transfusion	2	7.4	0	...	2	5.2
Condyloma	1	3.7	0	...	1	2.6
Chronic renal insufficiency	1	3.7	0	...	1	2.6
Total	27	100	11	100	38	100

TABLE 4. HBV serologic markers other than HB_sAg found in HB_sAg-positive study women.

Results	Anti-HB _s Ag		HB _e Ag		Anti-HB _e Ag		Anti-HB _c Ag	
	No.	%	No.	%	No.	%	No.	%
Positive	3	4.5	16	21.3 ^a	49	65.3	65	90.3
Negative	64	95.5	59	78.7	26	34.7 ^b	7	9.7
Total	67	100	75	100	75	100	72	100

^a 95% CI = 1.04-30.56
^b 95% CI = 23.93-45.47

every 5-10 years, because of the potential decline in protective antibody levels over time (12).

Considering that 9.5% of those found positive in the screening test for HB_sAg at the time of delivery were not confirmed by confirmatory testing, it would appear logical to focus research efforts on the prenatal period, thus avoiding both passive and active immunoprophylaxis in unconfirmed cases and markedly reducing costs. However, the low utilization of prenatal services by women in Brazil constitutes a constraint upon implementation of such a strategy.

As previously noted, the observed prevalence of HBV carriers (HB_sAg seropositives) was significantly higher among women whose pregnancies terminated in miscarriage (1.84%) than among those who delivered live infants (0.83%). The clinical hospital attended by the study population sees a high number of miscarriages induced by low-income women who are

also exposed to relatively high risks of contracting sexually transmitted diseases—a circumstance that could account for the higher observed HBV infection rates among the study women who miscarried.

The small percentage (27.6%) of infected women in whom HBV risk factors or behaviors were identified by anamnesis is in accord with most of the relevant studies in the literature (15, 19)—a circumstance indicating that no such epidemiologic measure can replace serologic screening. Part of the discrepancy observed is associated with omission of information about situations and behaviors currently being repressed by society, such as use of illicit drugs and sexual promiscuity (29). Another partial explanation is that many patients may not know that their partners are or have been exposed to high risk of infection.

While hepatitis is not as discrimination-provoking as the acquired immunodeficiency syndrome (AIDS), the

two diseases share several common features. For instance, the current study found intravenous drug use (a major AIDS risk factor) to be the risk factor most frequently encountered among women infected with HBV. (Other leading risk factors were a history of hepatitis, jaundice, and HIV infection.) Again as in the case of AIDS, alcoholism was considered a risk factor, because alcoholics are more prone than nonalcoholics to intravenous drug use and sexual promiscuity.

With regard to serologic markers of HBV infection other than HB_sAg, the finding of anti-HB_cAg in 65 (90.3%) of the samples from 72 of the 76 women repeatedly positive for HB_sAg suggests that the test for this marker could be used as an alternative in screening for maternal HBV infection. Special attention should also be given to the fact that 16 (21.3%) of the 75 HB_sAg-positive samples tested were found positive for HB_eAg.

Even though some authors recommend universal screening of all obstetric populations for HB_s antigens (30), it is important to stress that routine screening yields a better cost-benefit situation when the infection is endemic or when a selective screening program fails to isolate a significant number of HBV carriers among the general population. Population data for the United States indicate that universal screening appears to be cost-effective (considering both direct and indirect costs) when the prevalence of the carrier state exceeds 0.06% (31). However, in developing countries it is essential to balance the cost-efficiency aspects and the potential impact of a screening policy aimed at pregnant women against other primary health care problems that continue unresolved.

The World Health Organization (WHO) recommends that for populations with medium to high prevalences (exceeding 2%) of chronic HBV infection, appropriate intervention should consist of early vaccination of the newborn—since transmission occurs primarily among lactating and very young babies. In such circumstances, the vaccination schedule

should begin shortly after birth, principally in hyperendemic areas (where the prevalence of chronic HBV exceeds 10%). In areas with prevalences below 2%, WHO recommends including the option of screening pregnant women and actively immunizing the newborn babies of infected mothers, realizing that simultaneous administration of immunoglobulin against hepatitis B could present operational and cost problems (13).

In the specific obstetric population studied, even though the prevalence of HBV-positive women (0.95%) may be considered low, the high observed prevalence of HB_eAg carriers and the failure to detect anti-HB_eAg antibodies in 34.7% of 75 who were confirmed positive for HB_sAg points to a significant opportunity for vertical transmission of the virus. Virtually all those born to mothers carrying HB_eAg will be infected at birth, and 90% of the infected babies will become chronic carriers (32-34). If one considers that some 70% of the infections in the confirmed positive study women would not have been detected through a routine clinical anamnesis (which would have permitted maternal-child HBV transmission intervention at the earliest opportunity), universal screening for HB_sAg would appear justifiable in this population. Since the cost of each routine screening test is approximately

US\$ 5.00, as compared to US\$ 99.00 for the three-dose vaccination series, such screening would provide an alternative to routine vaccination of all newborns. At present, obstetric screening at the time of delivery is operationally feasible in the study community, despite the additional costs and effort implied by the screening test's 9.5% false-positive rate. It should be noted, however, that routine vaccination could become a more economical alternative if mass vaccination of infants were implemented in Brazil and if vaccination costs were reduced, which has not yet happened.

Maximum protection against vertical transmission of HBV is achieved when a combination of hyperimmune HBV human immunoglobulin (HBIG) and vaccine is administered shortly (not more than 48 hours) after birth. This treatment has a protective efficacy superior to 90-95%, even when the mother carries the HB_e antigen and does not have anti-HB_e antibodies (33). Use of hepatitis B vaccine alone for prevention of vertical transmission has rates of protective efficacy in the range of 70-85%, depending on the frequency of the HB_eAg marker in the obstetric population (35). Considering that no anti-HB_e antibodies were detected in 26 (34.7%) of 75 women confirmed positive for HB_sAg, the safest intervention for this popula-

tion was general inclusion of HBIG in all cases of neonatal prophylaxis. This procedure was adopted in the study and continues to be used at the hospital. Due to its high cost, however, it is not a measure that could easily be extended to all regions of the country.

We believe that this study has made an important contribution to the existing pool of information about the prevalence of HBV carriers and corresponding serologic markers among obstetric populations in Southeastern Brazil. Dissemination of its findings should help to alert epidemiologists, obstetricians, and neonatologists about the need to conduct assessments of their patients in their communities and to allocate resources for planning public health measures at the regional level. On the basis of these findings, it is proposed that routine screening of HBV carriers be instituted as early as possible by means of HB_sAg detection, followed by both active and passive immunoprophylaxis when economic and operating conditions permit. The early immunization of all newborn infants, as is proposed for developed countries (36), is still costlier than serologic screening of pregnant women and seems to us unsuited to the present reality of Brazil and other Latin American countries.

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RESUMO

Frequência de gestantes portadoras do HB_sAg em uma comunidade brasileira

Visando aferir a taxa de reatividade sérica do HB_sAg e de outros marcadores da infecção pelo VHB em parturientes, além de avaliar quais os fatores de risco, estudaram-se 7992 mulheres que tiveram suas gestações resolvidas no Hospital das Clínicas da Universidade de São Paulo. A reatividade sérica do HB_sAg foi aferida por o teste ELISA em duas etapas: a primeira com período de incubação de 2 h (triagem) e a segunda de 18 horas (confirmatória) realizada nas amostras positivas ao triagem. Os marcadores anti-HB_sAg, HB_eAg, anti-HB_eAg, e anti-HB_cAg foram testados nas amostras confirmadamente positivas. No triagem, foram positivas para o HB_sAg 1,05% (IC95%; 0,84-1,30) das amostras. Apenas 0,95% (IC95%; 0,75-1,19) foram confirmadamente positivas, sendo significativamente superior nas pacientes cujas gestações terminaram em aborto (1,84%) do que as 0,84% que terminaram em parto (X^2 correção de Yates = 7,76; $P < 0,005$). A anamnese possibilitou identificar fatores de risco para a infecção pelo VHB em apenas 27,6% das pacientes avaliadas. Dessas amostras, 21,3% (IC95%; 1,04-30,56) apresentavam HB_sAg positivo, de elevado risco para transmissão vertical desse vírus. Esses resultados reforçam a necessidade de pesquisa sorológica específica no final da gestação, possibilitando o máximo benefício da imunoprofilaxia neonatal.