# Serosurvey of hepatitis B surface antigen in pregnant Saudi women

*M.K.M. Khalil,[1] Y.Y. Al-Mazrou,[2] M. Al-Jeffri,[2] Y.S. Al-Ghamdi,[3] A. Mishkhas[2] M. Bakhsh,[2] M. Eisa,[2] M. Nageeb[2] and S. Tumsah[2]*

## مسح مصلي للمستضد السطحي لالتهاب الكبد البائي لدى الحوامل السعوديات

محمد خليل، يعقوب المزروع، محمد الجفري، ياسر الغامدي، أمين مِشْخَص، محمد بَخْش، مصطفى عيسى، محمد نجيب، صلاح تمساح

**الخلاصة:** بدأت المملكة العربية السعودية في عام 1990 تطعيم جميع الأطفال عند سن المدرسة ضد التهاب الكبد البائي. وقد قام الباحثون بتقييم معدل انتشار المستضد السطحي لالتهاب الكبد البائي بين الحوامل السعوديات، بعد ذلك باثنتي عشرة سنة في خمس مناطق بالمملكة، باستخدام أسلوب العينة المتعددة المراحل. وقد شاركت 2664 سيدة سعودية حاملاً في الدراسة المستعرضة التي أجريَتْ، وتم فحص عينات من الدم للكشف عن وجود المستضد السطحي لالتهاب الكبد البائي، كما تم فحص العينات الإيجابية لتحرِّي وجود المستضد الغلافي لالتهاب الكبد البائي. ولقد كانت النتيجة إيجابية للمستضد السطحي في 2.44٪ من السيدات المشتركات في البحث، كما كانت إيجابية أيضاً للمستضد الغلافي في أربع سيدات (0.15٪) للمستضد الغلافي لالتهاب الكبد البائي. وكان أعلى معدل لانتشار المستضد السطحي لالتهاب الكبد البائي في منطقة جيزان (4.2٪) وأدنى معدّل انتشار في منطقة تبوك (1.4٪). وكانت نسبة الإيجابية لدى النساء تحت سن العشرين 0.5٪، مقارنةً بـ 2.6٪ لدى النساء اللواتي هنّ أكبر سناً (P = 0.049). وكان المعدل الإجمالي لانتشار التهاب الكبد البائي أقل مما كان عليه من قبل.

ABSTRACT In 1990, Saudi Arabia began vaccinating all children at school entry against hepatitis B. We evaluated hepatitis B surface antigen (HBsAg) prevalence rate among pregnant Saudi women 12 years later in 5 regions of the country. Using multistage sampling, 2664 pregnant Saudi women were recruited. Blood samples were tested for HBsAg; positive samples were also tested for hepatitis Be antigen (HBeAg). In all 2.44% were positive for HBsAg and 4 (0.15%) were also positive for HBeAg. HBsAg prevalence was highest in Gizan (4.2%) and lowest in Tabuk (1.4%). Positivity for women < 20 years of age was 0.5% compared with 2.6% for older women (***P*** = 0.049). The overall HBsAg prevalence rate was lower than previously reported.

**Enquête sérologique sur l'antigène de surface du virus de l'hépatite B chez les femmes enceintes saoudiennes**

RÉSUMÉ En 1990, l'Arabie saoudite a commencé à vacciner contre l'hépatite B tous les enfants à l'entrée à l'école. Nous avons évalué le taux de prévalence de l'antigène de surface du virus de l'hépatite B (Ag HBs) chez les femmes enceintes saoudiennes 12 ans plus tard dans 5 régions du pays. En utilisant la technique d'échantillonnage à plusieurs degrés, 2664 femmes saoudiennes enceintes ont été recrutées. Des échantillons de sang ont été soumis à un test de dépistage de l'Ag HBs ; des échantillons positifs ont également été soumis à un test de dépistage de l'antigène e du virus de l'hépatite B (Ag HBe). En tout, 2,44 % des femmes étaient positives pour l'Ag HBs et 4 (0,15 %) étaient également positives pour l'Ag HBe. La prévalence la plus élevée de l'Ag HBs était observée à Gizan (4,2 %) et la plus faible à Tabuk (1,4 %). La positivité pour les femmes de moins de 20 ans était de 0,5 % contre 2,6 % pour les femmes plus âgées (p = 0,049). Le taux global de prévalence de l'Ag HBs était inférieur à ce qui était indiqué précédemment.

*[1]Research Unit, Medical Education and Research Centre, King Fahd Specialist Hospital, Qassim, Saudi Arabia (Correspondence to M.K.M. Khalil: statkhl@hotmail.com).*
*[2]Ministry of Health, Riyadh, Saudi Arabia.*
*[3]Riyadh Medical Complex, Riyadh, Saudi Arabia.*

## Introduction

Globally, an estimated 350 million people are chronic hepatitis B virus (HBV) carriers. The virus claims over 1 million lives each year due to the progress of chronic disease to cirrhosis or hepatocellular carcinoma [*1*]. Chronic HBV infection with persistence of hepatitis B surface antigen (HBsAg) occurs in 90% of infants infected by perinatal transmission, in 30% of children 1–5 years of age infected after birth, and in 5%–10% of older children, adolescents and adults with HBV infection. Chronically infected individuals are at high risk of developing chronic liver disease, such as cirrhosis, chronic active hepatitis or chronic persistent hepatitis, or primary hepatocellular carcinoma later in life [*2*].

High prevalence of HBsAg and hepatitis Be antigen (HBeAg) in pregnant women is the most important factor contributing to the high carrier rate of HBsAg. Several factors, including age at infection, predispose to the acquisition and frequency of the carrier state [*3*]. Vaccination of the neonates of infected mothers can interrupt 85%–90% of mother–infant transmissions. However, the transmission of HBV through the placenta within the uterus cannot be interrupted by hepatitis B vaccination and intrauterine transmission is an important mode of spreading HBV infection. Reported rates of intrauterine transmission vary from 4.4% to 23.5% but are mostly in the range of 5%–15% [*4,5*].

In Saudi Arabia, the HBsAg carrier rate was 16.7% for adults and 6.7% for children in a baseline survey before mass immunizations began in 1990 [*6,7*]. The rate varied with region and population. For example, it was 11.1% for children in Gizan in the south-west and as high as 19.9% for adult Saudi males [*8,9*]. At the same time, a high incidence of primary heptocellular carcinoma was reported in the country, especially in Gizan where the prevalence of HBsAg carrier status was high [*10,11*].

Maternal–fetal transmission of hepatitis B virus *in utero* or during the perinatal period has not appeared to be important in maintaining the carrier state in Saudi Arabia [*12*]. Prevalence of HBeAg in asymptomatic Saudi mothers is low compared with asymptomatic carrier mothers from the Middle East [*13*]. This may explain the minor role of vertical transmission in Saudi Arabia [*12,13*].

Universal hepatitis B vaccination in infancy was implemented in Saudi Arabia in 1990 to prevent early acquisition of infection. At the same time, another programme was launched to vaccinate all schoolchildren at school entry as a second target group. It was expected that by the year 2002, 12 years after the start of the programme, this vaccination strategy would have led to the protection of all Saudis aged 0–18 years who are at higher risk of acquiring hepatitis B virus and of developing the chronic carrier state. Knowing that the segment of the population under the age of 15 years was 46.2% of the total population and that those aged 15–19 years were 10.9% of the population, over 55% of the population would then be covered by the vaccination in the year 2002 [*14*].

After the mass vaccination era, the considereable effect of the mass immunization programme on the seroepidemiolgy of HBV infection in Saudi Arabia was reported [*15*]. The overall HBsAg carrier rate in children dropped from 6.7% in 1989 to 0.3% in 1997 [*15*]. The full effect of the vaccine on pregnant Saudi women will take time to be seen, that is until more of the vaccinated cohorts reach the age of marriage. The carrier state of this important group should be evaluated not only as an indicator of when to shift hepatitis B vaccine from infancy to later in life, but also to compare vaccinated

and unvaccinated cohorts, monitor regional variation and develop regional policies if needed.

As 12 years has passed since the school entry vaccination programme began, we aimed to evaluate the HBsAg prevalence rate among pregnant Saudi women and to identify age and regional variations.

## Methods

We conducted a cross-sectional study with a planned sample of 2910 pregnant Saudi women from the 5 main regions in Saudi Arabia: Northern, Southern, Eastern, Western and Central regions. Multistage sampling techniques were used to draw the sample. Sample size was determined so as to calculate the national prevalence of the HBsAg positivity and to detect with 80% power and 95% confidence interval the differences in the prevalence rates of each region.

Within each of the country's 5 regions, 1 health province was selected randomly. They were: Tabuk (Northern), Gizan (southern), Al-Gatif (eastern), Madina (western) and Al-Qassim (central). From each province, we selected 12 primary health care centres, 8 from urban areas and 4 from rural areas. The number of pregnant Saudi women selected from each province was dependent upon the population size of the province in relation to the total population of Saudi Arabia (Table 1). A total of 241 women in Madina dropped out probably because the study took place during the haj (pilgrimage) when there are many people on the move and this adversely affected recruitment.

Blood samples (5 mL) were collected in plain tubes at the primary health care centres. All sera samples were stored in duplicate at –20 °C and transported frozen in cold boxes to the Department of Virology in the Central Laboratory and Blood Bank, Riyadh. Then they were assayed for HBsAg. Positive samples were tested also for HBeAg.

Table 1 **Regional distribution of pregnant Saudi women**

| Region | Planned No. | Enrolled[a] No. | Dropped No. | Tested[b] No. |
|---|---|---|---|---|
| Gatif | 400 | 400 | 0 | 398 |
| Madina | 840 | 599 | 241 | 596 |
| Qassim | 840 | 840 | 0 | 840 |
| Gizan | 550 | 550 | 0 | 550 |
| Tabuk | 280 | 280 | 0 | 280 |
| Total | 2910 | 2669 | 241 | 2664 |

***[a]Enrolled = planned – dropped.***
***[b]Tested = no. of samples processed in the laboratory. Differences between this and the number enrolled were due to inadequate sample or other technical reasons.***

Enzyme-linked immunosorbent assay (ELISA) for HBsAg and HBeAg screening and HBsAg confirmatory test (neutralization) were applied with HBsAg screening version 3, HBsAg confirmation version 3 and HBeAg/Anti-HBe (Abbott Murex, Dartford, Kent, United Kingdom).

Data were analysed with *SPSS*, version 10. Analysis of variance (ANOVA) was used to compare means and standard deviations (SD) and the chi-squared test was used to compare qualitative data. $P < 0.05$ was considered significant.

## Results

Table 2 shows the mean age, number of pregnancies and proportion under 20 years of age of the sample by region. The mean age was 28.44 (SD 6.76) years with a regional variation of 29.42 years in Qassim and 27.39 years in Gatif ($P = 0.0001$). There were significant regional differences

Table 2 **Mean age and number of pregnancies of the 2664 pregnant Saudi women and the proportion under the age of 20 years of age**

| Demographic characteristic | Gatif | Madina | Qassim | Gizan | Tabuk | Total | *P*-value |
|---|---|---|---|---|---|---|---|
| ***Age (years)*** | | | | | | | |
| Mean (SD) | 27.39 (6.02) | 28.71 (6.95) | 29.42 (6.67) | 27.79 (7.14) | 27.72 (6.48) | 28.44 (6.76) | 0.0001 |
| No. | 396 | 581 | 836 | 544 | 279 | 2636[a] | |
| ***Pregnancies per woman*** | | | | | | | |
| Mean (SD) | 3.56 (2.75) | 4.48 (3.19) | 3.97 (3.1) | 4.84 (3.79) | 4.82 (3.31) | 4.29 (3.28) | 0.0001 |
| No. | 380 | 528 | 824 | 531 | 279 | 2542 | |
| ***Women < 20 years*** | | | | | | | |
| % | 5.8 | 5.7 | 5.6 | 10.8 | 8.6 | 7.1 | 0.001 |
| No./total no. | 23/396 | 33/581 | 47/836 | 59/544 | 4/279 | 186/2636 | |

*[a]Missing data for age = 28 (2664–2636).*
*SD = standard deviation.*

in the number of pregnancies and the proportion of pregnant women under the age of 20 years. The overall proportion of pregnant women under the age of 20 years was 7.1%. Of the girls who had been vaccinated in 1990 during the first year of primary school, the oldest were now 18–19 years of age.

The mean age at first pregnancy was 22.7 (SD 4.1) years with a significant regional variation from 23.5 years in Qassim to 21.2 years in Tabuk ($P = 0.0001$). This may reflect older age at marriage in Qassim.

In total, 2664 women were screened and 84 were positive for HBsAg. After HBsAg confirmation, only 65 were positive. Accordingly, 2.4% of the total sample were HBsAg positive with a 95% confidence interval of 2.11%–2.69%. Regionally this varied from 4.2% in Gizan to 1.4% in Tabuk ($P = 0.035$) (Table 3). Only 4 of the 65 HBsAg carriers were positive for HBeAg, i.e. 0.15% (4/2664) of the total sample or 6.15% of the HBsAg carriers.

Only 1 case was positive for HBsAg among women under the age of 20 years (1/186), i.e. 0.5% positivity rate compared with 2.6% for the older age group ($P = 0.085$ for two-sided test and $P = 0.049$ for one-sided test). This woman was 19 years of age whereas the first cohort vaccinated at school entry in 1990 was approximately 18 years of age.

Surgical procedures were associated with higher HBsAg positivity (3% or 15/449), but this was not significant. HBsAg positivity and dental procedures or blood transfusions were also not significantly associated (Table 4).

Table 5 shows HBsAg positivity by age group. The prevalence rate was significantly lower among women aged 20–39 years than for older women ($P = 0.0001$). Assuming an overall prevalence of HBsAg of 7% in the pre-vaccination era, Figure 1 shows the effect of age on HBsAg prevalence expressed as the percentage decrease of HBsAg prevalence.

Table 3 **HBsAg and HBeAg positivity rates by region**

| Positivity | Gatif | Madina | Qassim | Gizan | Tabuk | Total | *P*-value |
|---|---|---|---|---|---|---|---|
| ***HBsAg positive*** | | | | | | | |
| % | 2.5 | 2.3 | 1.7 | 4.2 | 1.4 | 2.4 | 0.035 |
| No. | 10/398 | 14/596 | 14/840 | 23/550 | 4/280 | 65/2664 | |
| ***HBeAg*** | | | | | | | |
| % | 0.5 | 0.2 | 0 | 0.2 | 0 | 0.15 | 0.28 |
| No. | 2/398 | 1/596 | 0/840 | 1/550 | 0/280 | 4/2664 | |
| ***Positive women < 20 years*** | | | | | | | |
| *%* | 0 | 0 | 2.1 | 0 | 0 | 0.5 | 0.65 |
| No./total no. | 0/23 | 0/33 | 1/47 | 0/59 | 0/24 | 1/186 | |

## Discussion

The year 2002 marked the 12th anniversary of the implementation of the mass hepatitis B vaccination in Saudi Arabia. Since 1990, substantial progress has been made towards eliminating HBV transmission in children. Accordingly, the incidence of HBV has substantially declined among groups with high vaccination coverage, such as young children. The relative burden of HBV-related diseases acquired from infections in childhood, however, still exists. Adult carriers still pose a risk to their unvaccinated contacts.

Table 4 **HBsAg positivity rate by risk factor for HBV infection**

| Risk factor | HBsAg positivity rate % | No./total no. | *P*-value |
|---|---|---|---|
| ***Under 20 years of age*** | | | |
| Yes | 0.5 | 1/186 | |
| No | 2.6 | 64/2450 | |
| Total | 2.5 | 65/2636 | 0.049 (one-sided) |
| ***History of operation*** | | | |
| Yes | 3.0 | 15/499 | |
| No | 2.3 | 50/2163 | |
| Total | 2.4 | 65/2662 | 0.224 |
| ***Blood transfusion*** | | | |
| Yes | 1.7 | 3/174 | |
| No | 2.5 | 62/2490 | |
| Total | 2.4 | 65/2662 | 0.7 |
| Dental procedures | | | |
| Yes | 2.3 | 32/1408 | |
| No | 2.6 | 33/1256 | |
| Total | 2.4 | 65/2662 | 0.64 |

Table 5 **Prevalence of HBsAg in pregnant Saudi women by age group**

| Age group (years) | No. of women screened | No. positive | % (95% confidence interval) |
|---|---|---|---|
| < 19 | 186 | 1 | 0.53 (0.47–1.6) |
| 20–24 | 663 | 14 | 2.11 (1.21–3.61) |
| 25–29 | 674 | 16 | 2.37 (1.41–3.9) |
| 30–34 | 539 | 13 | 2.41 (1.35–4.2) |
| 35–39 | 382 | 8 | 2.09 (0.98–4.25) |
| 40+ | 192 | 13 | 6.77 (3.8–11.5) |
| Total | 2636 | 65 | 2.47 (1.92–3.15) |

In the early 1980s, the prevalence of HBsAg among pregnant Saudi mothers was determined. Prevalence rates ranged from 3.9% to 16%, which is higher than in our study [*10,11*]. This difference cannot be explained by the mass immunization programme alone, as most of the sample population had not been exposed to it. However, it could be explained by the adoption of universal precaution for the handling of blood

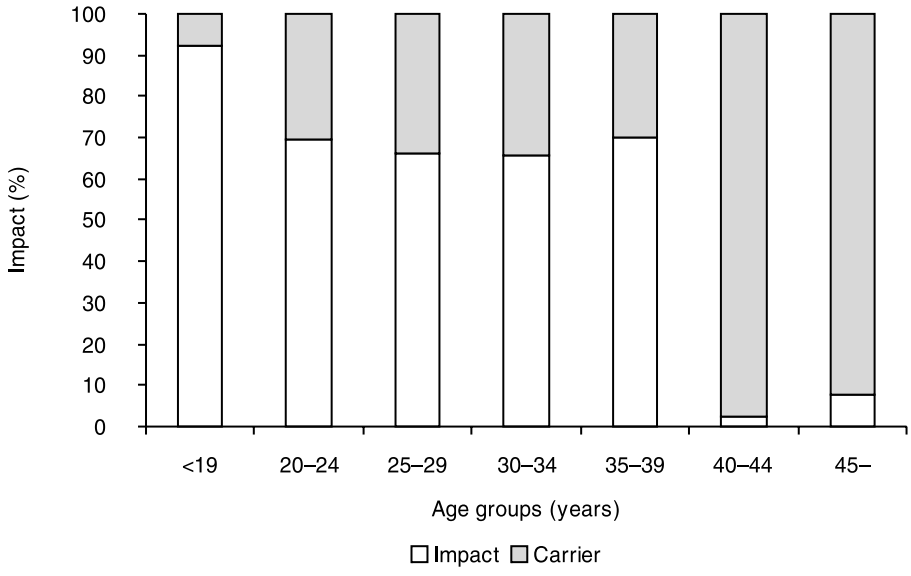


Figure 1 **Direct and indirect effect of age on HBsAg prevalence, assuming a norm of 7% positivity for all age groups during the pre-vaccination era (expressed as a per cent of the previous norm)**

and human fluids and excretion, thus decreasing exposure to the virus. For example, in our study, no increased risk was found between the carrier rate and exposure to known risk factors such as dental procedures, surgical operations and blood transfusions (Table 4).

Over 50% of the Saudi population has now been vaccinated with the hepatitis B vaccine, which decreases the risk of exposure to the unvaccinated. The indirect effect of vaccination and the adoption of standard universal procedures were more evident among ages 20–39 years than among older pregnant women (Table 5, Figure 1). There was a 70% decrease in HBsAg prevalence among ages 20–39 years compared with more than 90% among women under age 20 years and compared with less than 10% among women 40 years and older. For our evaluations, we used 7% as the overall prevalence of HBsAg in Saudi Arabia during the prevaccination era [*7,10,11*]. This rate is conservative, but a reasonable figure to use so as not to overestimate the effect of immunization.

The women who as children had participated in the hepatitis B vaccination programme had not yet fully reached childbearing age. This was evident when comparing mothers under 20 years with the remainder of the sample. The positivity rate was 0.5% for those under 20 years of age and 2.6% for older women ($P = 0.085$ for two-sided test and 0.049 for one-sided test). Only 1 of 186 women under 20 years was positive: she was 19 years of age whereas the oldest cohort vaccinated at school entry in 1990 was around 18 years of age during our study.

Regional variation was evident, and Gizan had the highest prevalence rate at 4.2% (Table 3). These regional differences need to be addressed by policy-makers.

The national strategy to eliminate HBV in Saudi Arabia is based primarily on vaccinating all infants. Screening all pregnant women is not part of this strategy, as vertical transmission has not been identified as an important route of infection in local studies [*10,11,16*]. Similarly screening pregnant mothers with the purpose of preparing immunoglobulin to be given with the vaccine at birth to infants of positive mothers is not cost-effective. Currently, however, the elimination programme needs to be accelerated to include additional interventions. Thus Saudi mothers could be vaccinated during their childbearing years, at least on a regional basis in, for example, Gizan where the infection rate is higher than in other regions. In addition, pregnant mothers should be screened and appropriate preventive measures adopted. Vaccinations should be considered for mothers who test negative.

We will have to wait a few more years until the vaccinated cohort reaches the age of marriage and childbearing in Saudi Arabia to fully evaluate the hepatitis B immunization programme. By then, the prevalence of HBsAg among pregnant Saudi mothers should have reached a minimum and it should be sufficient to shift the first dose of hepatitis B vaccine to a later age instead of at birth. Then combined vaccines containing hepatitis B could be given with diptheria/pertussis/tetanus (DPT) and *Haemophilus influenzae* type-b.

## Acknowledgements

This study was supported by GlaxoSmith-Kline, Riyadh. The authors acknowledge with deep gratitude the effort of the physicians and nurses in the regional primary health care centres and hospitals for their efforts during fieldwork.

## References


1. Maddrey WC. Hepatitis B: an important public health issue. ***Journal of medical virology***, 2000, 61:362–6.
2. ***Red Book: Report of the committee on infectious Diseases, 24th ed***. Elk Grove Village, Illlinois, USA, American Academy of Pediatrics, 1997:247.
3. Vranckx R, Alisjahbana A, Meheus A. Hepatitis B virus vaccination and antenatal transmission of HBV markers to neonates. ***Journal of viral hepatitis***, 1999, 6(2):135–9.
4. Stevens CE et al. Perinatal hepatitis B virus transmission in the United States. ***Vaccine***, 1998, 16:S48–51.
5. Qian X et al. [Monitoring of mother-child transmission of hepatitis B virus during pregnancy using molecular hybridization methods.] ***Zhonghua fu chan ke za zhi***, 1992, 27:259–62.
6. El-Hazmi MA. Hepatitis B virus in Saudi Arabia. ***Journal of tropical medicine and hygiene***, 1989, 92(1):56–61.
7. Al-Faleh FZ et al. Seroepidemiology of hepatitis B virus infection in Saudi children: a baseline survey for mass vaccination against hepatitis B. ***Journal of infection***, 1992, 24(2):197–206.
8. Parande CM, Arya SC, Ashraf SJ. Hepatitis B virus among Saudi children in Gizan, Saudi Arabia. ***Infection***, 1986, 14(5):223–5.
9. Arya SC et al. Hepatitis B virus in Gizan, Saudi Arabia. ***Journal of medical virology***, 1985, 17(3):267–74.
10. Atiyah M, Ali MA. Primary hepatocellular carcinoma in Saudi Arabia. A clinicopathological study of 54 cases. ***American journal of gastroenterology***, 1980, 74(1):25–9.
11. Ashraf SJ et al. A profile of primary hepatocellular carcinoma in the Gizan area of Saudi Arabia. ***Cancer***, 1986, 58(9): 2163–8.
12. Bsalamah AH, Serebour F, Kazim E. Materno–foetal transmission of hepatitis B in Saudi Arabia. ***Journal of infection***, 1984, 8(3):200–4.
13. Ramia S et al. Vertical transmission of hepatitis B surface antigen in Saudi Arabia. ***Annals tropical paediatrics***, 1984, 4(4):213–6.
14. ***Population characteristics in the Kingdom of Saudi Arabia, demographic survey, 1999***. Riyadh, Central Department of Statistics, Ministry of Planning, 1999.
15. Al-Faleh FZ et al. Seropepidemiology of hepatitis B virus infection in Saudi children 8 years after mass hepatitis B vaccination programme. ***Journal of infection***, 1999, 38(3):167–70.
16. Zamir C et al. Evaluation of screening for hepatitis B surface antigen during pregnancy in a populatin with high prevalence of hepatitis B surface antigen-positive/hepatitis B e antigen-negative carriers. ***Pediatric infectious disease journal***, 1999, 18(3):262–6.