

Chronic HBV infection in pregnant immigrants: a multicenter study of the Italian Society of Infectious and Tropical Diseases

Evangelista Sagnelli¹, Gloria Taliani², Francesco Castelli³, Dario Bartolozzi⁴, Bruno Cacopardo⁵, Orlando Armignacco⁶, Gaetano Scotto⁷, Nicola Coppola⁸, Tommaso Stroffolini⁹, Caterina Sagnelli¹⁰

¹Infectious Diseases, Second University of Naples, Italy; ²Infectious Diseases, University of Rome La Sapienza, Italy;

³Infectious Diseases, University of Brescia Italy; ⁴Infectious Diseases, S. M. Annunziata di Firenze Hospital, Florence, Italy;

⁵Infectious Diseases, University of Catania, Italy; ⁶Infectious Diseases, Belcolle Hospital, Viterbo, Italy;

⁷Infectious Diseases, Ospedali Riuniti of Foggia, Italy; ⁸Infectious Diseases, Second University of Naples, Italy;

⁹Infectious Diseases, University of Rome La Sapienza, Italy; ¹⁰Infectious Diseases, Second University of Naples, Italy

SUMMARY

The aims of the study were to estimate the clinical impact of HBV infection in pregnant immigrants and their family members and to identify a useful approach to managing the healthcare of HBsAg-positive immigrants.

Included in this study were 143 HBsAg-positive pregnant immigrants of the 1,970 from countries with intermediate/high HBV endemicity who delivered in 8 Italian hospitals in 2012-2013. In addition, 172 family members of 96 HBsAg-positive pregnant immigrants were tested for serum HBsAg.

The median age of the 143 HBsAg-positive pregnant immigrants was 31.0±12.1 years and the length of stay in Italy 5.0±4.1 years; 56.5% were unaware of their HBsAg positivity. HBV DNA was detected in 74.5% of the pregnant immigrants, i.e., 94.3% from Eastern Europe, 72.2% from East Asia and 58.1% from Sub-Saharan Africa. HBV DNA ≥2000 IU/mL was detected in 47.8% of pregnant immigrants, associated with ALT ≥1.5 times the upper normal value in 15% of cases. Anti-HDV was detected in 10% of cases. HBsAg was detected in 31.3% of the 172 family members. All HBsAg-positive immigrants received counseling on HBV infection and its prevention, and underwent a complete clinical evaluation.

The findings validate the approach used for the healthcare management of the HBsAg-positive immigrant population.

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INTRODUCTION

As a consequence of socio-economic and political crises in Northern and Sub-Saharan Africa, Eastern Europe, the Middle-East and East Asia in recent years, Italy has become a land of immigration from these geographical areas. In spite of the intermediate or high endemicity level of hepatitis B virus (HBV) infection in their countries of origin, the majority of immigrants coming to Italy are unaware of their HBV serological status. Once in Italy, this immigrant population, prevalently young and with insufficient income, is socially disintegrated due to language, cultural and socio-economic barriers and has limited access to the healthcare services (Coppola *et al.*, *in press*; Fasano *et al.*, 2013; Kim, *et al.*, 2014; Majori *et al.*, 2008; Sagnelli C, *et al.*, 2014; Sagnelli C *et al.*, 2015; Sagnelli E,

et al., 2014b,c; Sagnelli *et al.*, 2012a; Spada *et al.*, 2011). Pregnancy and the future delivery of the baby, however, induce pregnant immigrants to refer to a hospital of the Italian National Healthcare System, where screening for serological markers of HBV infection is always performed in accordance with the strategies of the universal anti-HBV prophylaxis of newborn babies in Italy. Only a few studies have been published to date on chronic HBV infection in pregnant immigrants in Italy, mostly concerning the results of screening for serum hepatitis B antigen (HBsAg) at delivery, with HBsAg prevalences ranging from 2.5% to 5.9%, most probably reflecting a different composition of the study samples investigated (Bonura *et al.*, 2005; Spada *et al.*, 2011; Stroffolini *et al.*, 2003). To the best of our knowledge, no further informative investigation has been reported more recently. The immigrant population has changed a great deal in Italy because of an increased silent massive immigration from East Asia, Ukraine and Romania and because of wars involving countries in Northern and Sub-Saharan Africa and the consequent increase in the number of refugees from these geographical areas.

This paper analyzes the demographic, laboratory and

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Corresponding author:

Evangelista Sagnelli

E-mail: evangelistasagnelli@libero.it

clinical data of the 143 HBsAg-positive pregnant immigrants of the 1,970 who delivered in 2012/2013 at 8 Italian hospitals in different cities in Northern, Central and Southern Italy (Brescia, Florence, Rome, Viterbo, Caserta, Naples, Foggia, Catania).

The paper also describes the first screening performed in Italy to detect HBV infection in family members of the HBsAg-positive pregnant immigrants. The HBsAg-positive pregnant immigrants and their HBsAg-positive family members were counselled on the prophylactic measures to prevent the transmission of HBV infection and were referred for clinical investigation free of charge to the unit of infectious diseases of the hospital where immigrant women delivered. All newborn babies underwent HBV immunoprophylaxis.

MATERIALS AND METHODS

The present study regards the 143 consecutive HBsAg-positive pregnant women out of the 1,970 pregnant immigrants observed during pregnancy or at the time of delivery in 2012/2013 at one of the 8 hospitals in different cities in Northern, Central and Southern Italy (Brescia, Florence, Rome, Viterbo, Caserta, Naples, Foggia, Catania). These pregnant migrants came from countries with an intermediate or high HBV endemicity, mostly from Northern and Sub-Saharan Africa, Eastern Europe, the Middle East and East Asia.

For each pregnant immigrant a pre-coded questionnaire reporting the demographic, epidemiological, laboratory and clinical data was filled out by the physician in care at the first observation. One hundred and seventy-two family members of 96 HBsAg-positive pregnant immigrants were screened for HBsAg on a voluntary basis. All HBsAg-positive pregnant immigrants and their HBsAg-positive family members were referred for a clinical and, if necessary, therapeutic follow-up at the Unit

of Infectious Diseases of the hospital where the women delivered.

The study was approved by the Ethics Committee of the Azienda Ospedaliera Sant'Anna e San Sebastiano of Caserta (number protocol 222/2013). All the procedures used in the study complied with the standards on human experimentation of the relative Ethics Committees and with the Helsinki Declaration of 1975, revised in 1983. Each subject enrolled in the present study signed their informed consent in accordance with the rules of the local Ethics Committees.

Serum samples were tested for HBsAg, hepatitis B e antigen (HBeAg) and for the antibody to HBeAg (anti-HBe) by commercial enzyme immunoassays (Abbott Laboratories, North Chicago, IL, USA). The antibody to the hepatitis delta antigen (HDV-Ab) was sought using a commercial enzyme immunoassay (DiaSorin, Saluggia, VC, Italy). Plasma HBV DNA was sought by real-time polymerase chain reaction as previously described (Coppola *et al.*, 2010; Coppola *et al.*, 2013). Liver function tests were performed using routine methods. Continuous variables were summarized as mean and standard deviation, and categorical variables as absolute and relative frequencies. Differences in the mean values were evaluated by the Student t-test, and the chi-squared test was applied to categorical variables. A p value <0.05 was considered to be statistically significant.

RESULTS

Of the 143 HBsAg-positive pregnant immigrants investigated, 53 were from Eastern Europe, 35 from East Asia, 48 from Sub-Saharan Africa, 5 from Northern Africa and 2 from South America. The demographic characteristics of these 143 and of the three largest geographical subgroups are shown in *Table 1*. The median age was 31.0±12.1 years, with only slight differences in the three largest subgroups

Table 1 - Demographic and epidemiological characteristics of the 143 HBsAg-positive pregnant immigrants, and the data for the three largest subgroups.

	Total	Eastern Europe	East Asia	Sub-Saharan Africa
HBsAg positive, N.	*143	53	35	48
Age, years, M±SD	31±12.1	32.2±17.8	30.8±6	29.9±4.6
At their first pregnancy, N. (%)	80 (55.9)	36 (67.9) A	22 (62.9) C	15 (31.3) B D
Living in Italy for years, M±SD	5.0±4.1	5.4±3.9 E	7.3±5.3 F G	4.4±3.1 H
HBsAg positivity before pregnancy:				
known, N. (%)	61 (43.3)	19 (35.8) I	20 (60.6) J	20 (41.7)
unknown, N. (%)	80 (56.7)	34 (64.2)	13 (40.6)	28 (58.3)
missing, N.	2	0	2	0
HBsAg positivity before pregnancy, known for years, M±SD	6.0±5.6	5.9±6.7 K M	8.8±5.8 L	2.4±2.3 N
HBsAg positivity known before pregnancy, pregnancy:				
with chronic hepatitis B, N. (%)	20 (32.8)	8 (42.1)	4 (20)	6 (30)
asymptomatic carriers, N. (%)	41 (67.2)	11 (57.9)	16 (80)	14 (70)

*Only 5 pregnant immigrants were from Northern Africa and 2 from South America; consequently these subcontinents are not included in this table. A vs B: p=0.003; C vs D: p=0.003; E vs F: p=0.0001; G vs H: p=0.0001; I vs J: p=0.02; K vs L: p=0.04; M vs N: p=0.0001.

Table 2 - Laboratory data of the 143 HBsAg-positive pregnant immigrants, and the data for the three largest subgroups.

	Total	Eastern Europe	East Asia	Sub-Saharan Africa
HBsAg-positive pregnant women, N.	143*	53	35	48
HBsAg, N. (%):				
Positive	23 (19.3)	9 (20)	10 (38.5) A	3 (7.1) B
Negative	96 (80.7)	36 (80)	16 (61.5)	39 (92.9)
Missing, N.	24	8	9	6
HBsAb, N. (%):				
Positive	(65.7)	38 (82.6) C	15 (57.7) DE	38 (90.5) F
Negative	24 (16.8)	8 (17.4)	11 (42.3)	4 (9.5)
Missing, N.	25	9	9	6
HBV DNA, N. (%):				
Positive	73 (73.7)	33 (83.3) G	13 (72.2)	22 (51.3) H
Negative	26 (26.3)	3 (8.3)	5 (27.8)	17 (43.6)
Missing, N.	44	17	17	9
HBV DNA IU/mL, M±SD	1.3E7±5E7	1.2E7±4.2E7	3.9E7±8.8 E7	1.6E6±1.3E6
HBV DNA IU/mL, N. (%):				
≤2000 IU/mL	36 (52.2)	18 (58.1)	5 (38.4)	10 (50)
>2000 IU/mL	33 (47.8)	13 (41.9)	8 (61.5)	10 (50)
Missing, N.	4	2	0	2
ALT, IU/mL, M±SD	1.0±1.5	1.6±2.2	1.7±0.6	1.3±0.7

*Only 5 pregnant immigrants were from Northern Africa and 2 from South America; consequently, these subcontinents are not included in this table.

A vs B: $p=0.001$; C vs D: $p=0.02$; E vs F: $p=0.001$; G vs H: $p=0.0006$.

x u.n.v. times upper normal value.

(Table 1). The mean length of the stay in Italy was longer in pregnant women from East Asia (7.3 ± 5.3 years) compared to those from Eastern Europe (5.4 ± 3.9) or Sub-Saharan Africa (4.4 ± 3.1 , $p=0.0001$). Of the 143 pregnant women, 80 (55.9%) were at their first pregnancy, a prevalence higher in the Eastern Europe (67.9%) and East Asia (62.9%) subgroups than in the Sub-Saharan Africa (31.3%, $p<0.0001$) subgroup. Only 61 (43.5%) of the pregnant immigrants knew they were HBsAg chronic carriers at the time they were tested, a percentage higher in the East Asia subgroup (60.6%) than in those from Sub-Saharan Africa (41.7%) or Eastern Europe (35.8%, $p=0.02$) (Table 1). In addition, pregnant immigrants in the East Asia subgroup knew of their HBsAg positivity for longer than those in the Eastern Europe (8.8 ± 5.8 years vs 5.9 ± 6.7 , $p=0.04$) or Sub-Saharan Africa (2.4 ± 2.3 ; $p=0.0001$) subgroups. Also, the comparison between the Eastern Europe and Sub-Saharan Africa subgroups showed a statistically significant difference ($p=0.0001$) (Table 1). Twenty (32.8%) of the 61 pregnant immigrants who knew they were chronic carriers knew they had chronic hepatitis B, and 41 (67.2%) knew they were asymptomatic HBV carriers (Table 1). Table 2 shows the initial virological data of the 143 HBsAg-positive pregnant immigrants and of those in the three largest geographical subgroups. HBV DNA was detected in 73 (73.7%) of the 99 HBsAg-positive pregnant women tested, with wide variations in the geographical subgroups: 83.3% in the Eastern Europe subgroup versus 72.2% ($p<0.002$) in the East Asia

and 51.3% ($p<0.0006$) in the Sub-Saharan subgroup. Of the 73 HBV-DNA-positive pregnant immigrants, an HBV DNA serum level ≥ 2000 IU/mL, indicating active HBV replication, was detected in 47.8% of the cases, whereas lower levels suggestive of an inactive HBV carriage were detected in the remaining 52.2% (Table 2).

HBsAg was detected in 19.3% of the HBsAg-positive pregnant immigrants tested, with substantial differences in the geographical subgroups: 38.5% in those from East Asia versus 20% in those from Eastern Europe and 4% ($p=0.001$) in those from Sub-Saharan Africa (Table 2).

Due to the strong reduction in HDV endemicity in Italy in the latter two decades, antibodies to HDV-Ag are infrequently determined in Italian hospitals, as in hospitals in other western countries with low diffusion of HDV infection (Vergani *et al.*, 2008). In the present study, HDV-Ab was determined in nearly one third of the HBsAg-positive pregnant immigrants and detected in 10% of cases.

Each baby born of an HBsAg-positive immigrant mother started passive/active HBV immunoprophylaxis at birth, whereas those born of an HBsAg-negative mother started active immunoprophylaxis at the third month. One hundred and twenty-two family members of 96 HBsAg-positive pregnant immigrants were investigated, mostly husbands or children born of previous pregnancies, and 54 (31.4%) were found to be HBsAg positive. This prevalence ranged from 30 to 38.7% in the three largest geographical subgroups.

DISCUSSION

The number of pregnant immigrants harbouring chronic HBV infection observed in the present study calls for simplified access of this category to the clinical structures of the National Healthcare System not only for assistance in pregnancy and delivery, but also for their clinical assessment and follow-up. In fact, HBsAg-positive pregnant immigrants require a thorough evaluation of their clinical condition and in some cases long-term observation including possible anti-HBV treatment (Howell *et al.*, 2014; Nguyen *et al.*, 2014; Vallet-Pichard *et al.*, 2014). Delivery is an appropriate time to offer screening for HBV markers to the family members of the HBsAg-positive pregnant immigrants, nearly one third of whom were found to be HBsAg-positive in the present investigation. This emphasizes the strong epidemiological impact of vertical and family transmission of HBV in geographical areas with a high or intermediate HBV endemicity. This opportunity should not be missed since HBV chronic carriers are at risk of developing a severe liver disease and even hepatocellular carcinoma (HCC) (Howell *et al.*, 2014; Ladep *et al.*, 2014; Liu *et al.*, 2014), and of transmitting HBV infection to HBsAg/anti-HBs-negative individuals (Kubo *et al.*, 2014; Sagnelli *et al.*, 2012b; Sellier *et al.*, 2014).

As in the case of the HBsAg-positive pregnant immigrants, after a preliminary assessment of their clinical condition, all HBsAg-positive family members should receive counselling on HBV infection, its routes of transmission and its prevention. These HBsAg-positive family members should also undergo a clinical assessment and, when appropriate, a follow-up including possible anti-HBV treatment. Most of the HBsAg-positive pregnant immigrants in this study had detectable HBV DNA, a sign of HBV replication, half of them with HBV DNA serum levels ≥ 2000 IU/mL, a viral load considered to be associated with chronic liver disease.

In addition, one fifth of the HBsAg-positive pregnant immigrants in the present study were HBeAg positive, a clinical condition associated with a more frequent and rapid progression towards a severe liver disease and HCC (Sagnelli *et al.*, 1983; Vallet-Pichard *et al.*, 2014). The clinical presentation of our 143 HBsAg-positive pregnant immigrants is a strong warning for the Italian Healthcare Authorities to devise simplified access to allow HBsAg-positive immigrants to be followed up in qualified clinical centers, free of charge and with reduced bureaucratic procedures.

The limitations of this study are the moderate number of HBsAg positive mothers and of their family members investigated and the high number of missing for same laboratory data (HBeAg and HBV DNA), in part due to the moderate compliance of some pregnant women to be followed up at the Unit of Infectious Diseases of the hospital where they had delivered. The model used, however, allowed us to assess the clinical condition of 143 HBsAg-positive pregnant immigrants, 80 of whom were unaware of their HBsAg positivity, and of 53 HBsAg-positive family members, all unaware of their virological condition.

In addition, the model made it possible for all HBsAg-positive pregnant immigrants and their HBsAg-positive family members to have easy access to the clinic of infectious diseases of the hospital where the pregnant immigrants delivered, where they received counselling on HBV infection and its prevention and underwent a complete clinical

evaluation. The data from the present study strongly emphasize the need for active continuous screening to identify the HBsAg-positive subjects in the immigrant populations, particularly those from Eastern Europe and Sub-Saharan Africa, who more frequently than those from East Asia were unaware of their HBsAg positivity. Our model, validated in the present study, can be proposed to the Italian Healthcare Authorities for nationwide application.

In good agreement with international recommendations (Weinbaum CM 2008), the data from the present study emphasize the need for universal screening for HBV infection in people from countries with a HBsAg prevalence higher than 2% and remind the Italian healthcare authorities to carry out extensive screening and educational programs for these populations.

Conflict of interest:

All the authors of the manuscript declare that they have no conflict of interest in connection with this paper.

List of abbreviations:

Hepatitis B virus, HBV; hepatitis B antigen, HBsAg; hepatitis B e antigen, HBeAg; antibody to HBeAg, anti-HBe; antibody to hepatitis delta antigen, HDV-Ab; hepatocellular carcinoma, HCC.

Authors' contributions

ES, GT, FC, DB, BC, OA, GS, NC, TS, and CS contributed equally to this work. All the authors read the manuscript and gave approval of the final manuscript version to be published.

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