

A Seroprevalence Study for Hepatitis B Virus Markers of Infection in Pregnant Women in Romania: Results and Opportunities for Prevention

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ABSTRACT

Background & Aims: The Action Plan for viral hepatitis in the WHO European Region aims to eliminate them as a public health threat by the end of 2030. The hepatitis B virus (HBV) perinatal transmission is a problem of major concern. The aim of our study was to estimate the prevalence of HBV markers in pregnant women in Romania, as scientific evidence for recommending public health interventions.

Methods. The serum samples were prospectively collected in randomly selected maternities, from pregnant women admitted for birth beginning with July 1st, 2016. Signed informed consent was requested and the confidentiality of personal data was assured. The laboratory investigations were performed in two regional public health laboratories.

Results. The prevalence of HBsAg was 5.1%. Among HBsAg positives, the HBeAg was present in 7.4% and the anti-HBeAb were detected in 55.6% of the pregnant women.

Conclusion. The results of the study were comparable to those in 2013 for the women of the same age group, which proves the need for established public health interventions leading to reducing, and halting HBV transmission in the population.

Key words: Hepatitis B – seroprevalence – pregnant – evidence-based public health interventions.

Abbreviations: CDC: Centers for Disease Control and Prevention; HBIG: hepatitis B immune globulin; HBV: hepatitis B virus; HCV: hepatitis C virus; HCC: hepatocellular carcinoma; NIPH: National Institute of Public Health; VE: Vaccine effectiveness.

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INTRODUCTION

Globally, an estimated number of 325 million people were living in 2015 with hepatitis B virus (HBV) or C (HCV) chronic infections, and no less than 1.34 million died due to viral hepatitis the same year [1]. For people living in the European Union (EU) and the European Economic Area (EEA) countries, it was estimated that 4.7 million were chronically infected with HBV and 5.6 million with HCV. These infections are considered the major causes of chronic liver disease, liver cirrhosis and hepatocellular carcinoma (HCC) [2].

The Action Plan for the Health Sector Response to viral hepatitis in the World Health Organization European Region (WHO/EURO) [3] was finalized in 2017 and adapted by the Global Health Sector Strategy on Viral Hepatitis, 2016–2021 [4]. The aim of the Action Plan is to eliminate viral hepatitis as a public health threat in the region by the end of 2030. Elimination was defined as a 90% reduction in the number of new chronic hepatitis B and C infections and a 65% reduction in the number of deaths by 2030.

As mentioned in the “Strategic direction 2: Interventions for impact”, the HBV perinatal transmission is a problem of major concern because 70–90% of perinatally infected newborns become chronic carriers, being at high risk of evolution to cirrhosis, HCC and even death. In order to prevent the perinatal transmission, WHO/EURO adopted two main strategies: to ensure vaccination of all newborns with a dose of monovalent hepatitis B vaccine within 24 hours of birth, and the second is to screen all pregnant women for HBsAg prenatally, followed by post-exposure prophylaxis with hepatitis B vaccine birth dose and hepatitis B immune globulin (HBIG) for children born by carrier mothers.

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In Romania, antenatal screening for HBV is not a current policy. A single test is reimbursed to the family doctor by the National Health Insurance House, without specific recommendation for the month of pregnancy. The hepatitis B vaccination is assured by the National Vaccination Programme, with 4 doses given at birth, at 2, 4 and 11 months of age.

A first seroprevalence study for HVB markers of infection in pregnant women in Romania was performed in 1990 for the North-East Region, by a team of Romanian epidemiologists, in collaboration with CDC Atlanta [5]. The study revealed a 8.4% prevalence of HBs antigen (HBsAg), with the maximum value of 13.2% in the 35-45 years old age group, and a minimum of 6.6% in the 20-24 age group. It brought important public health information used as an argument for the implementation of hepatitis B universal childhood vaccination in Romania in 1995. The study performed in 1991 for the North-West Region, showed a 3.8% HBsAg prevalence in pregnant women [6]. This study was followed by another one, in 1995, on pregnant women in the South Region of Romania [7]. The HBsAg prevalence was 12.3%, significantly different from the 8.4% value registered in the first regional study [5]. The maximum value (20.8%) in the 1995 study [7] was observed in the 35-39 years age group, and the minimum one (7.7%) in ≥ 40 years.

The starting point for our study was the seroprevalence analysis for HBV and HCV infections, with national representativeness, performed by the National Institute of Public Health (NIPH) Romania in 2013, in all age groups and both genders [8]. The analysis revealed a 4.2% HBsAg prevalence in the study population. In women aged ≥ 20 years, the prevalence was 4.02%, while in the age group under 20, the calculated value was 1.37%. The 2.66% difference was statistically significant ($p = 0.02$). These values were used in the sample size calculation for this study.

The aim of the present study was to estimate the prevalence of HBV markers of infection in pregnant women in Romania, as scientific evidence for public health interventions. The objectives were to calculate the proportion of pregnant women having positive results for HBsAg, HBeAg and anti-HBeAb, overall and by age groups.

MATERIAL AND METHODS

Study design

The serum samples were prospectively collected in randomly selected maternities, from pregnant women admitted for birth starting with July 1st, 2016. The samples were leftover sera from other laboratory investigations. The signed informed consent of pregnant women was requested and the confidentiality of personal data was assured. The laboratory investigations were done in two regional public health laboratories of the NIPH Romania, using standardized ELISA kits.

Study population and sample size

For the pregnant women sample size calculation, the most recent official data (2014) for the new registered pregnant women in Romania were used: 129,723, out of which 13,329 were aged under 20 years and 116,394 ≥ 20 years (source: NIPH – NCSIPH).

We calculated the sample size using the EpiInfo 7 programme (Centers for Disease Control, CDC, Atlanta) for a national representativeness, an expected 4.02% HBsAg prevalence for pregnant women aged ≥ 20 years, and 1.37% for < 20 years, respectively, with a 2.5% precision of 95% CI. Adding 5% loss for each age group, we obtained a total number of 337 pregnant women, 87 aged < 20 years at the prelevation moment and 250 aged ≥ 20 years. In order to calculate the vaccine effectiveness with 1/1 ratio between the group a priori considered vaccinated (< 20 years old, with big vaccination coverage - around 95%) and the other one, also a priori considered not vaccinated (≥ 20 years old), we established a number of 250 pregnant women in each age group, with a total number of 500.

Selected maternities

We randomly selected 20 maternities (9%) from the state and private system, out of the total number (226) which existed in the National Electronic Vaccination Registry (RENV), using a counting step ($11 = 226/20$). We asked for 13 samples from both age groups to be selected from each maternity. Because of the low addressability to some maternities, we added 4 (24 in total), selecting from the list the next maternity existing in the same county.

Subjects

Inclusion criteria: pregnant women admitted for birth starting with July 1st, 2016, who had signed the informed consent form. *Exclusion criteria:* pregnant women admitted for birth before July 1st, 2016; pregnant women who did not sign the informed consent form; pregnant women already known with acute or chronic liver-biliary-pancreatic pathology. A single serum sample was obtained from each pregnant woman. The age was calculated for the date of sampling.

Vaccination history data

The vaccination status was searched at the family doctor, only for the HBsAg positive pregnant women, as well as the number of doses and the age when each dose had been received.

Study protocol approval and informed consent

The study protocol was approved by the Coordination Committee of the NIPH Romania. The informed consent was obtained from all the pregnant women participating in the study, and from their tutors, in case of minors, according to the World Medical Association Declaration of Helsinki, revised in 2000, Edinburgh. A copy of the signed document remained at the local public health authority, in order to further communicate the results to the participants in the study.

Samples' selection, transportation, storage and testing

The serum samples were selected from each maternity in the order of entry into the laboratory register, until the total number of samples has been reached. A unique protocol for transportation, storage and testing was followed. The tested markers of HBV infection were HBsAg, HBeAg and anti-HBeAb, the last two only for the positive HBsAg samples. The NIPH laboratories used the ELISA method (kits with 96-well microplate format), ELISA lines or automated analyzers including ELISA readers. For each marker the results

were communicated as "positive"/ "negative"/ "equivocal", in accordance with the manufacturer's specifications.

Data clearance, validation and analysis

We first verified the data completeness and accuracy, then analysed, using the Microsoft Excel 2010 programme, the following indicators: HBsAg prevalence (overall and by age groups); for the positive HBsAg women we calculated the HBeAg and the anti-HBeAb prevalence (overall and by age groups). Finally, we calculated the hepatitis B vaccine effectiveness (VE), using the formula proposed by the Centers for Disease Control (CDC) Atlanta [9]. For statistical significance we used EpiCalc 2000.

RESULTS

We collected 531 serum samples, 263 from pregnant women aged under 20 years, and 268 from those aged ≥ 20 years (Table I). The minimum age was 11 years and the maximum was 44, with a median of 20 years and an average of 23.

Table I. Prevalence of HBV infection markers in pregnant women, Romania, 2016

Age group (years)	<20 (n=263)	≥ 20 (n=268)	Overall (n=531)
HBsAg positive	10 (3.8%)	17 (6.3%)	27 (5.1%)
HBeAg positive	1 (0.4%)	1 (0.4%)	2 (0.4%)
anti-HBeAb positive	5 (1.9%)	10 (3.7%)	15 (2.8%)

HBsAg: surface antigen of HBV; HBeAg: "early" antigen = infectivity HBV marker; anti-HBeAb: antibodies against HBeAg

Regarding HBeAg as an infectivity marker of HBV, only one pregnant woman in each age group was positive, which corresponds to a 10% prevalence in age group under 20, of those HBsAg positive, and almost 6% for ≥ 20 years old. Using as a denominator the total number of tested women, the prevalence becomes 0.4% in each age group, and also overall. Even if the HBeAg prevalence in HBsAg positive pregnant women was almost double in the age group under 20, the difference was not statistically significant ($p = 0.71$). The two pregnant women with positive HBeAg had a negative result for anti-HBeAb.

More than a half of the HBsAg positive pregnant women had already had anti-HBeAb. All the anti-HBeAb positives were HBeAg negative. Two equivocal results for anti-HBeAb were communicated, which were not taken in prevalence calculation. No equivocal result for HBsAg and HBeAg was registered.

Vaccination status of pregnant women with positive HBV infection markers

For the 27 HBsAg positive pregnant women, the vaccination status was as follows: a number of 10 women (37%) had been fully vaccinated (3 doses), 8 beginning with birth age, and other 2 at 9 and 20 years old, respectively. The age of fully vaccinated women was between 15 and 28 years, with a median of 18 years. One pregnant woman had received a single dose of vaccine, and for another one the vaccination history was unknown.

Fifteen pregnant women (56%) had not been vaccinated. Fourteen of them (93%) belonged to the ineligible cohort for childhood vaccination (aged ≥ 20 years).

The age interval of unvaccinated pregnant women was 17 to 37 years, with a median of 28. A single woman, aged 17, belonged to the cohort eligible for childhood vaccination (<20 years old). The VE value was 40%.

DISCUSSION

Compared to the previous regional studies performed in Romania in pregnant women [5-7], our study had a national representativeness.

We found a 5.1% HBsAg overall prevalence, that exceeded with 0.4% the value registered in 2013 [8] for the same age interval, but the difference was not statistically significant ($p = 0.84$). In the age group ≥ 20 years the prevalence of HBsAg was 1.7 times higher than in the group <20 years, but the difference was also not statistically significant ($p = 0.25$). The HBsAg prevalence in age group < 20 years exceeded with 1.1% the value registered in 2013 [8] for the same age interval (11-19 years), again without statistical significance ($p = 0.74$). In age group ≥ 20 years, the same difference of 1.1% was observed compared to 2013, for 20-44 years old, with no statistical significance ($p = 0.57$).

Although the differences between the HBsAg prevalence observed in this study, and those registered in 2013 [8] were not statistically significant for different age groups, the higher absolute values demonstrated the necessity of firm public health interventions to reduce the HBV perinatal transmission in Romania. Out of the 8 pregnant women aged under 20, having positive HBsAg and being fully vaccinated, only 4 had been vaccinated according to the schedule. For the other 4, at least one dose had been delayed.

As limits for this study, we mention that our 2013 seroprevalence study [8], as a base for sample calculation, was performed on a hospitalized population and could have introduced a bias. The low addressability to some maternities could have caused a delay in scheduled activities. We did not use a stratified randomization of maternities, as the simple one had sufficient geographical representativeness, for different maternity sizes, and urban vs rural areas. We did not search the vaccination history for all the pregnant women participating in this study, but only for those HBsAg positive. The women aged under 20 years were a priori considered as belonging to a vaccinated cohort. This could have brought a bias in the VE calculation, by decreasing its value. As the samples were not randomly selected in each laboratory, we excluded from the study the already known positive pregnant women for HBV infection markers, to avoid the prevalence overestimation. From the HBV markers, we tested the HBsAg, HBeAg and anti-HBeAb, considered relevant for perinatal transmission. The viremic load was not an objective of this study, and could have been performed later in patients' management.

Testing pregnant women for HBsAg should be followed by testing of the positives for HBeAg, and especially for DNA-HBV [10]. The antiviral therapy should be initiated as soon as possible for the pregnant women with a DNA-HBV value >200,000 IU/ml, to reduce the perinatal transmission [10]. The

Table II. The post-exposure prophylactic measures recommended by the UK NSC for the newborn, based on mother's HBV status [12]

Mother's HBV status	The newborn should receive	
	Hepatitis B vaccine	HBIG
Positive HBsAg and HBeAg	Yes	Yes
Positive HBsAg, negative HBeAg and negative anti-HBeAb	Yes	Yes
Acute Hepatitis B during pregnancy	Yes	Yes
Positive HBsAg and positive anti-HBeAb	Yes	No
Positive HBsAg and DNA-HBV $\geq 1 \times 10^6$ IU/ml at any prenatal test done during this pregnancy (regardless of the HBeAg and anti-HBeAb result)	Yes	Yes
Positive HBsAg and birthweight ≤ 1500 g	Yes	Yes

post-vaccination testing of children born to HBsAg positive mothers should be done at 1-2 months after the complete 3 doses vaccination, but not before the age of 9 months, to avoid the detection of HBsAb existing in the specific HBIG administered the first hours after birth, and of the HBsAg from vaccine which could be positive 1-18 days after vaccination. The post-vaccination testing at a minimum age of 9 months of children born to positive HBsAg mothers, increases at maximum the probability to detect the HBV late infection [10]. The Advisory Committee on Immunization Practices (ACIP) recommends for the prematures having a birth weight < 2000 g, born to HBsAg negative mothers, to receive the first dose at one month of age or at discharge [11].

Since 1998, the screening of pregnant women for HBV and the prompt post-exposure vaccination of the newborns was recommended in the UK [12]. The HBV infected mothers should have a confirmatory test, followed by testing for "e" markers and viral load. An urgent HBsAg testing should be done in the case of unregistered mothers who are in labor, to ensure that the first dose of hepatitis B vaccine is given within 24 hours of birth. The "e" HBV markers or the viral load should also be quickly tested, to alert the need of HBIG administration (Table II).

In France, the recommended vaccination schedule for the children born to positive HBsAg mothers is 0, 1, 6 months, using a monovalent vaccine [13]. The first dose is administered at birth, in association with HBIG. For the prematures born before 32 weeks and with a birthweight under 2000 g, 4 doses of vaccine are recommended, at 0, 1, 2, 6 months.

In Romania it would be desirable to implement the second WHO strategy for perinatal HBV transmission prevention, regarding the pregnant women screening and post-exposure prophylaxis. We suggest that given the high values of HBsAg prevalence observed in our study, the HBsAg prenatal screening should be recommended for the first and the third trimester of each pregnancy, simultaneously with the screening for syphilis [14]. The confirmatory ELISA test should be followed by testing for the "e" markers and the viral load, to correctly assess the need for HBIG (Table II).

HBsAg positive pregnant women should be followed-up by a hepatologist or infectionist. They should have caesarean section [15, 16] and ab lactation recommendation in the presence of cracked or bleeding nipples [17], apart from when HBIG is given in due time to the newborn. Breastfeeding should be allowed only after using a nipple pump, in the absence of nipple rhagades. For HBV transmission preventive measures

to the newborn from a positive HBsAg mother, the first dose of monovalent vaccine should be given within 24 hours of birth, then completing the vaccination schedule at 1 and 6 months. It would be necessary to provide HBIG in the first 12 hours from discharge. The children born to mothers with an unknown HBsAg status should receive the first dose of hepatitis B vaccine within 24 hours of birth. The mother should be tested for HBsAg as soon as possible; in the case of a positive result, the newborn should receive HBIG as soon as possible. For the prematures born by positive HBsAg mothers before 32 weeks of pregnancy or with a birthweight under 2000 g, a 4 doses vaccination schedule at 0, 1, 2, and 6 months would be appropriate [13]. Scientific evidence showed that the immunological memory remained intact for a 30 year period in healthy persons with vaccination initiated after 6 months of age. The booster dose is not necessary after a complete schedule received in time in the case of immune-competent persons, but only for those immune-compromised, based on serological monitoring [18, 19].

Children aged 3-23 months born by HBsAg positive mothers should be carefully monitored according to the NIPH viral hepatitis B and C surveillance methodology [20], to detect early the occurrence of a perinatal acute hepatitis B.

CONCLUSIONS

The prevalence of HBsAg in Romanian pregnant women (5.1%) is comparable to that established in 2013, representing scientific evidence to recommend public health interventions to reduce perinatal HBV transmission. The fact that 93% of the positive HBsAg and unvaccinated pregnant women belonged to ineligible cohorts for vaccination at birth is an argument for childhood vaccination.

Conflicts of interest: None to declare.

Authors' contributions: O.P. coordinated the study, collected the data from regional level and undertook the data analysis. R.R. and A.P. contributed to the methodology, coordinated the activities at regional level, participated in drafting the manuscript. D.A. performed a mentorship activity, participated in drafting the manuscript. All authors approved the current version of the manuscript.

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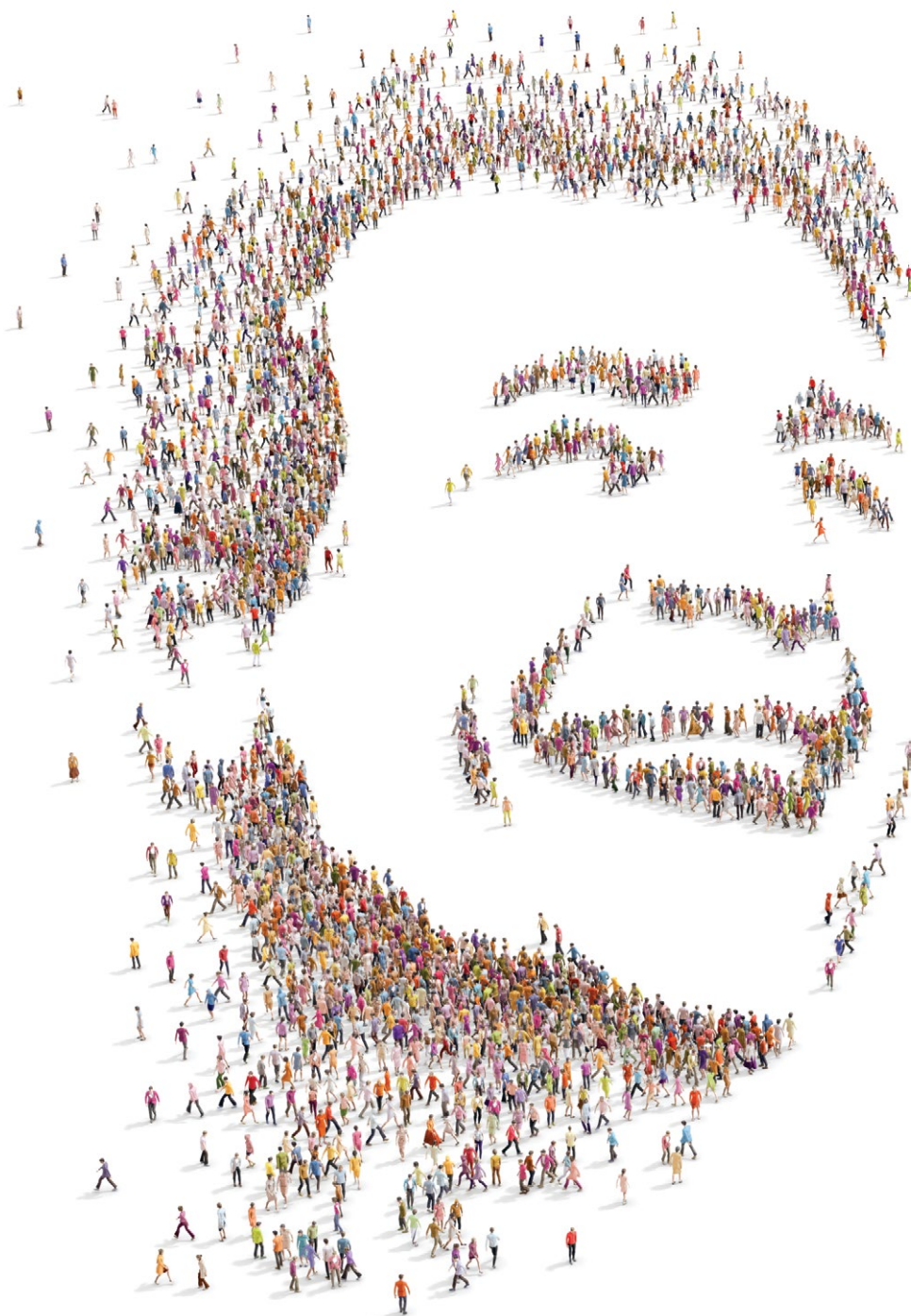
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