

**Screening for HBsAg, HCV and HIV among pregnant women,
Istria County, 2011-2012***Testiranje trudnica na biljege virusnog hepatitisa B i C te HIV infekcije u
Istarskoj županiji tijekom 2011. i 2012. godine***Jasmina Kučinar, Tatjana Vilibić-Čavlek, Bernard Kaić, Branko Kolaric***

Summary

During a two year period (2011 - 2012), a total of 1,596 consecutive serum samples from pregnant women were tested for the presence of HBsAg, anti-HCV and anti-HIV antibodies. HBsAg was detected in 11/1596 (0.7%; 95% CI = 0.34 - 1.23) tested women. All of them were HBeAg negative/anti-HBe positive. According to age, HBsAg prevalence was highest in the age group of less than 26 years (1.9%). In the age groups 26-30 and 31-35 years, prevalence rates of 0.5% and 0.4% were found. Differences among age groups were not significant ($p = 0.096$). Comparing age groups, a significantly lower prevalence of HBsAg was found in 31 - 35 age group compared to the youngest age group (< 26 years) (OR = 0.19, 95% CI = 0.04-0.97). Anti-HCV antibodies were detected in 12/960 tested women (1.3%, 95% CI = 0.64-2.17). HCV seropositivity increased significantly with age from 0.3% to 3.1% beginning with the 26-30 age group ($p = 0.03$). Place of residence was not associated with HBsAg (0.8% in urban areas vs. 0% in rural areas, $p = 0.616$) or anti-HCV positivity (1.8% in urban vs. 1.2% in rural areas, $p = 0.641$). None of the 1,002 participants were HIV positive. The results of this study suggest that Croatia is a low-prevalence country for HBV, HCV and HIV infection.

Key words: HBsAg, HCV, HIV, prevalence, pregnant women

Sažetak

Tijekom dvogodišnjega razdoblja (2011. – 2012. godine), testirano je ukupno 1596 uzoraka seruma trudnica na prisutnost HBsAg, anti-HCV i anti-HIV protutijela. HBsAg je dokazan u 11/1596 (0,7%; 95% CI = 0,34 - 1,23) testiranih žena. S obzirom na dob, prevalencija HBsAg bila je najviša (1,9%) u skupini mlađih od 26 godina. U dobi od 26-30 i 31-35 godina bilo je pozitivno 0,5%, odnosno 0,4% ispitanica. Razlike između dobnih skupina nisu bile statistički značajne ($p = 0,096$). Uspoređujući dobne skupine, nađena je značajno niža prevalencija HBsAg u skupini od 31 - 35 godina u usporedbi s najmlađom dobnom skupinom (< 26 godina) (OR = 0,19; 95% CI = 0,04 - 0,97). Anti-HCV protutijela nađena su u 12/960 testiranih žena (1,3%; 95% CI = 0,64 - 2,17). Opažen je značajan porast HCV seropozitiviteta s dobi od 0,3% do 3,1%, počevši s dobnom skupinom od 26-30 godina ($p = 0,03$). Ovisno o mjestu prebivališta (urbano ili ruralno područje), nije bilo značajne razlike u prevalenciji HBsAg (0,8% u urbanoj te 0% u ruralnoj sredini; $p = 0,616$) niti anti-HCV (1,8% u urbanoj, te 1,2% u ruralnoj sredini; $p = 0,641$). Niti jedna ispitanica nije bila HIV pozitivna. Rezultati ovoga istraživanja ukazuju na nisku prevalenciju HBV, HCV i HIV infekcije u Hrvatskoj.

Key words: HBsAg, HCV, HIV, prevalencija, trudnice*Med Jad 2014;44(1-2):45-50*

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Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections represent an important public health problem worldwide.¹ Vertical transmission from infected mother to child is a common route of HBV transmission. It is widely accepted that most perinatal transmission of HBV occurs at or near the time of birth, because neonatal vaccination prevents newborn infection in about 80% - 95% of cases.² The risk of perinatal HBV transmission is reported to be 70 - 90% for infants born to mothers who are HBsAg/HBeAg positive, and it decreases to 5 - 20% for infants born to HBsAg/anti-HBe positive mothers.³ Worldwide, 30 - 50% of chronic HBV infections could be attributed to maternal-infant transmission.⁴ Since perinatal transmission of HBV results in a high frequency of chronic infection (up to 90%), early recognition of HBsAg positive pregnant women helps in identifying newborns at risk of perinatal HBV transmission who need postexposure-immunoprophylaxis.⁵

The acquisition of HCV infection through perinatal transmission is estimated to occur in about 1 - 6% of infants born to HCV-infected mothers, with the risk being proportional with the level of the mother's viremia.⁶ Moreover, most studies have reported a higher risk of perinatal HCV transmission in infants born to HIV co-infected mothers.^{7,8} Since there is no effective method for preventing vertical HCV transmission, routine prenatal screening for HCV infection is not currently advised. However, screening of high-risk women is important because antiviral therapy could be considered in the postpartum period that may decrease their risk of end stage liver disease and hepatocellular carcinoma.⁹

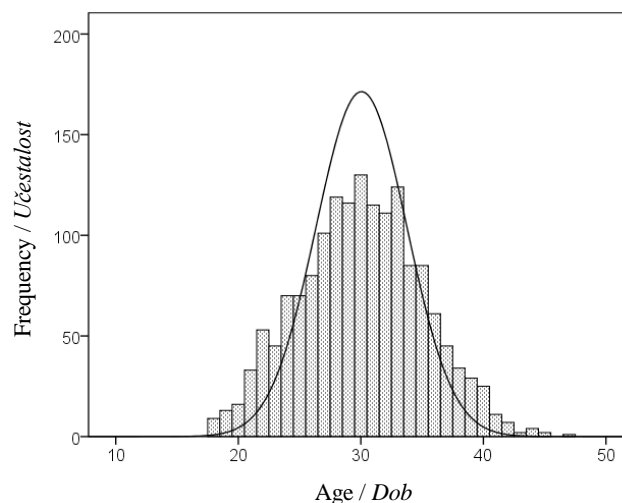
More than 90% of HIV infections in children are caused by perinatal transmission.¹⁰ The risk of perinatal HIV could be reduced from 15 - 40% to less than 2% if an accurate diagnosis of the mother's HIV infection is made and appropriate treatment provided.^{11,12}

Seroprevalence of HBV, HCV and HIV differs among countries as well as among different regions and populations in the same country. The aim of this study was to evaluate the prevalence of HBsAg, anti-HCV and anti-HIV among pregnant women in Istria County, Croatia.

Materials and methods

The study area was Istria County, the largest Croatian peninsula located in the north-western part of the Adriatic Sea. Istria County has in total 208,055 inhabitants with about 1,400 deliveries per year.

During a two-year period (2011 - 2012), a total of 1,596 consecutive serum samples from pregnant women aged 18-47 years (Picture 1) attending antenatal polyclinics were tested for the presence of HBsAg, anti-HCV and anti-HIV antibodies. HBsAg testing of pregnant women is mandatory, while anti-HCV and anti-HIV tests were performed on the request of the referring physician. HBsAg was detected using commercial enzyme-linked fluorescent assay (ELFA). Repeatedly reactive samples were further analyzed using confirmation test with neutralizing antibodies (Mini Vidas, bioMérieux, Marcy l'Etoile, France). HBsAg positive samples were additionally tested for the presence of HBeAg, anti-HBe, anti-HBs, anti-HBc total antibodies and anti-HBcIgM using ELFA (Mini Vidas, bioMérieux, Marcy l'Etoile, France).



Picture 1 Age distribution of study participants
Slika 1. Raspodjela učesnika studije prema dobi

Anti-HCV antibodies were screened by third generation enzyme-linked immunoassay (Ortho HCV Version 3.0 ELISA Test, Ortho-Clinical Diagnostics, Raritan, NY, USA). Initially reactive samples were confirmed by third generation line immunoassay (INNO-LIA HCV Score, Innogenetics, Ghent, Belgium).

HIV screening was performed by commercial ELISA for detection of HIV1/HIV2 IgG antibodies (HIV 1&2 Ab, Dialab GmbH, Neudorf, Austria). Borderline reactive samples would have been tested by fourth generation ELFA with simultaneous detection of p24 Ag; HIV1/HIV2 IgG antibodies (Mini Vidas, bioMérieux, Marcy l'Etoile, France).

Statistical analysis

Differences between groups of variables were assessed using Fisher’s exact test for categorical variables and Mann-Whitney U test for continuous variables. The strength of association between outputs (HBsAg, anti-HCV) was assessed using binary logistic regression. The software used for this analysis was Stata/IC ver. 11.2, StataCorp LP.

Results

HBsAg was detected in 11/1596 (0.7%; 95% CI = 0.34-1.23) tested pregnant women. All HBsAg carriers were positive for anti-HBc total antibodies and anti-HBe while anti-HBs and IgM anti-HBc antibodies were negative. According to age, HBsAg prevalence varied from 0.4% to 1.9% with no

significant difference between age groups (p = 0.096) (Table 1). There was no seropositive woman above 36 years of age. Comparing seroprevalence by age groups using the youngest age group as a reference group, a significantly lower seroprevalence was found in 31-35 age group compared to the youngest age group (< 26 years) (OR = 0.19, 95% CI = 0.04-0.97). The median age of HBsAg positive women was significantly lower than the median age of HBsAg negative women (25 vs. 30 years, p = 0.007) (Table 3).

Anti-HCV antibodies were detected in 12/960 tested pregnant women (1.3%, 95% CI=0.64-2.17). A significant increase in anti-HCV positivity with age was observed from 0.3% to 3.1% beginning with the 26-30 age group (p = 0.03). There was no HCV seropositivity detected in women younger than 25 and older than 40 years of age (Table 2).

Table 1 Prevalence of HBsAg in pregnant women, Istria County, Croatia

Tablica 1. Učestalost HBsAg kod trudnica, Istarska županija, Hrvatska

Characteristics <i>Karakteristike</i>	Tested / <i>testirane</i> N (%)	HBsAg positive / <i>pozitivno</i> N (%)	95% CI	OR	95% CI (OR)
<i>Age group (years) / Dobne skupine</i>					
< 26	309 (19.4)	6 (1.9)	0.71-4.18	1	N/A*
26-30	546 (34.2)	3 (0.5)	0.11-1.60	0.28	0.07 - 1.12
31-35	520 (32.6)	2 (0.4)	0.05-1.38	0.19	0.04 - 0.97
36-40	194 (12.1)	0	N/A -1.88	N/A	N/A
40+	27 (1.7)	0	N/A -12.77	N/A	N/A
<i>Place of residence / Mjesto boravka</i>					
Rural / <i>ruralno</i>	161 (10.1)	0	N/A -2.27	N/A	N/A
Urban / <i>urbano</i>	1435 (89.9)	11 (0.8)	0.38-1.37	N/A	N/A

*N/A = not applicable / *ne primjenjuje se*

Table 2 Prevalence of anti-HCV antibodies in pregnant women, Istria County, Croatia

Tablica 2. Učestalost anti-HCV antitijela kod trudnica, Istarska županija, Hrvatska

Characteristics <i>Karakteristike</i>	Tested / <i>testirane</i> N (%)	anti-HCV positive / <i>pozitivno</i> N (%)	95% CI	OR	95% CI (OR)
<i>Age group (years) / Dobne skupine</i>					
< 26	167 (17.4)	0	N/A*- 2.18		
26-30	322 (33.5)	1 (0.3)	0.01-1.7**	1	N/A
31-35	332 (34.6)	7 (2.1)	0.85-4.29	6.91	0.85-56.51
36-40	127 (13.2)	4 (3.1)	0.86-7.87	10.44	1.16-94.32
40+	12 (1.3)	0	N/A -26.46	N/A	N/A
<i>Place of residence / Mjesto boravka</i>					
Rural / <i>ruralno</i>	112 (11.7)	2 (1.8)	0.22-6.3	1	N/A
Urban / <i>urbano</i>	848 (88.3)	10 (1.2)	0.57-2.16	0.66	0.14-3.03

*N/A = not applicable/ *ne primjenjuje se* ** p = 0.030

The median age of anti-HCV positive women was significantly higher than the median age of anti-HCV negative women (34 vs. 30 years, $p = 0.003$) (Table 3).

The place of residence was not associated with HBsAg (0.8% in urban areas vs. 0% in rural areas, $p = 0.616$) or anti-HCV positivity (1.8% in urban vs. 1.2% in rural areas, $p = 0.641$) (Table 1 and 2). None of the 1,002 tested participants were anti-HIV borderline or positive.

Table 3 Median age of HBsAg positive/negative and anti-HCV positive/negative participants

Tablice 3. Prosječna dob HBsAg negativne/pozitivne te anti-HCV pozitivne/negativne učesnice

Serologic markers <i>Serološki markeri</i>	Median age (years) <i>Proječna dob</i>	p
HBsAg		0.007
Positive / <i>Pozitivno</i>	25 (18 - 33)	
Negative / <i>Negativno</i>	30 (18 - 47)	
Anti-HCV		0.003
Positive / <i>Pozitivno</i>	34 (29 - 40)	
Negative / <i>Negativno</i>	30 (18 - 45)	

Discussion

The overall HBsAg prevalence found in Istria County (0.7%) is comparable to that reported in France (0.65%)¹³ and in Gijón, Spain (0.8%).¹⁴ In some European countries lower prevalences were reported: 0.1% in Norway,¹⁵ 0.1% in Catalonia, Spain,¹⁶ 0.26% - 0.4% in Denmark^{17,18} and 0.48% in Germany.¹⁹ HBsAg positivity rates were higher in Italy (1%),²⁰ Turkey (2.8%)²¹ and Greece (2.9%-3.9%)^{22,23} and very high in Albania (7.3%).²⁴ There are few data on the HBsAg prevalence among pregnant women in Croatia. Two studies conducted in 1995 and 1999 showed seropositivity of 1% in the Istria and Rijeka regions²⁵ and 0.75% in Dalmatia.²⁶ In 2011, the overall HBsAg prevalence based on information from 31 maternity hospitals was 0.2%, with a range from 0.0% to 1.3%. The prevalence of HBsAg in women giving birth in Pula Maternity Hospital (Istria County) was 0.5% (data from the CNIPH). The difference in HBsAg seroprevalence in pregnant women in Istria County found in this study and the one reported by Pula Maternity Hospital for 2011 indicates that the populations are different. Namely, all women who were tested for HBsAg in our study did not necessarily give birth in Pula hospital and women who come to give birth in Pula could have been tested elsewhere (private laboratories or public health laboratories in other counties). In the

future, we can expect a further decrease in HBsAg prevalence among pregnant women, since universal hepatitis B immunization of 12-year olds was introduced in 1999 and consequently, the pool of susceptible young adults is decreasing.

Until 1990, intrafamilial transmission and IDU were the main risk factors for HBV infection. In most recent years, risk sexual behaviour, in particular multiple sexual partners, has been predominant.²⁰ From among 11 HBsAg positive pregnant women in this study, two were household contacts of HBsAg carriers, two had multiple hospitalizations with surgical procedures, one had pierced her ears in childhood, one had a history of blood transfusion in 1992 and one had sexual contacts with a promiscuous partner. The remaining four women did not report any identifiable risk factor for HBV infection.

As in France, where the highest HBsAg positivity was observed in pregnant women before the age of 20,¹³ the highest percentage of positive women in our study was recorded before the age of 26 (1.9%). This group includes the women who should have been vaccinated against HBV and we expected lower HBsAg positivity. However, four participants (out of six positive in this group) in the youngest age group belong to the Roma ethnic group, in which the vaccination program is not implemented regularly and completely. Three of them had uncontrolled pregnancies.

There are very few data on the prevalence of HCV in pregnant women in Croatia. In this study, we found the overall anti-HCV prevalence of 1.3%, which is similar to that reported from Gijón, Spain (1.44%).¹⁴ In Europe, lower prevalences were reported in the United Kingdom (0.19%-0.22%)²⁷ and Scotland (0.3%-0.4%).²⁸ Italy, Greece and Slovakia reported higher seropositivity rates (1.9%, 0.8-1.95% and 2.2%, respectively).^{20,23,29,30}

In a previous Croatian study (2003-2006), lower prevalence of HCV among pregnant women (0.5%) was found than in our study.³¹ Injection risk behavior is considered to be the predominant mode of HCV infection.³² In Croatia, the overall prevalence of HCV among intravenous drug users (IDUs) is reported to be 51%.^{33,34} A similar prevalence of HCV (49%) was found among pregnant IDUs.³⁵ Of 12 anti-HCV positive pregnant women in this study, 10 reported a history of IDU while one reported a former relationship with an IDU. Like British authors,²⁷ we found a significant increase in HCV seropositivity with age.

In this study, we found no HIV seropositive women which reflects the very low prevalence of HIV infection in the Croatian general population (< 0.1%).³⁶

The limitation of this study is a sample size, which resulted in relatively wide confidence intervals, which should be taken into account at interpretation of the results. Seroprevalence studies in some other population groups such as occupationally exposed groups would shed more light to understanding the epidemiology of these infections, in addition to already studied prevalence in high-risk groups.

In conclusion, the results of this study seem to confirm observations from earlier studies and routine data collection which indicate that Croatia is a country with a low prevalence of HBV, HCV and HIV infection.

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