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Seroprevalence and Risk Factors for HIV, Hepatitis B, and Syphilis in Populations with High-risk Behaviors in Croatia

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ABSTRACT HIV and sexually-transmitted diseases (STDs) represent a significant public health problem worldwide. We analyzed the seroprevalence and risk factors for HIV, hepatitis B and syphilis in populations with high-risk behaviors in Croatia. During a three-year period, a total of 443 men who have sex with men (MSM) / bisexual persons, sex workers (SW) / clients of SW, persons with multiple sexual partners, and persons with a history of STD were tested for the presence of HIV, hepatitis B virus (HBV), and *Treponema pallidum* (syphilis) antibodies within the framework of second generation HIV surveillance. Participants were recruited from 11 Croatian counties, the vast majority among clients of voluntary counselling and testing centers. The overall prevalence of HIV, HBsAg, anti-HBc, and syphilis was 1.4%, 2.6%, 12.1%, and 3.4%, respectively. HBV and syphilis seroprevalence differed significantly between genders with higher prevalence among men (anti-HBc 13.8% vs. 5.7%, $P=0.043$; syphilis 4.4% vs. 0%; $P=0.049$), as well as between age groups, with a steady increase according to age. Participants with a history of STD were more often seropositive than participants who did not report STD (HBsAg 8.2% vs. 1.0%, $P=0.002$; anti-HBc 32.4% vs. 6.4%, $P<0.001$; syphilis 12.0% vs. 1.7%, $P<0.001$). Syphilis seroprevalence was higher in homo / bisexual persons (12.2%) compared with heterosexual persons (1.2%, $P<0.001$). Logistic regression showed that history of STD was a significant risk factor for hepatitis B (HBsAg AOR=6.229, 95% CI=1.491-26.022; anti-HBc AOR=5.872, 95% CI=2.899-11.896) and syphilis seropositivity (AOR=5.572, 95% CI=1.751-17.726), while homo / bisexual behavior was associated with syphilis seropositivity (AOR=12.820, 95% CI=3.688-44.557). Our results highlight the importance of continuing STDs screening and prevention in at-risk populations.

KEY WORDS: HIV, hepatitis B, syphilis, high-risk populations, seroprevalence, Croatia

INTRODUCTION

HIV and sexually-transmitted diseases (STDs) continue to be a significant public health problem worldwide. The World Health Organization estimates that

about 37 million of the world population is chronically infected with human immunodeficiency virus (HIV), 350 million with hepatitis B virus (HBV) (1), and

about 18 million become infected with *Treponema pallidum* (syphilis) every year (2). Prevalence varies by geographic region and population group. Men who have sex with men (MSM) and commercial sex workers (CSW) remain high-risk groups for acquisition of HIV and other STDs, due to continuing high risk sexual behaviors such as multiple sexual partners, inconsistent condom use and co-infection with another STDs (3-7). In heterosexual persons, factors associated with sexual transmission include unprotected sex with an infected partner or with multiple sexual partners, as well as a history of another STD (8,9).

Although incidence of HIV decreased globally in recent years, the HIV epidemic in MSM and bisexual persons continue to progress in most countries (10-12). In Europe, HIV seroprevalence among MSM is reported to be 1-17% (13,14). In CSW, seroprevalence differed greatly between countries and even within the same country (1.08-24.70%) (15,16). HBV has been recognized as an important STD among MSM and CSW for many years (17), of whom 3.2-27.1% showed serologic evidence of HBV infection (anti-HBc antibodies) (14,18,19) and <1-7% had chronic infection (18-21). In addition to sexual risk factors, injecting drug use (IDU) is still an important risk factor for HBV transmission (8).

Over the past decade, syphilis has re-emerged in many European countries, mostly due to increased incidence among MSM with high risk sexual behavior (6). Seroprevalence rates of syphilis among MSM and CSW varied from 0.5% to a high 36.9% (16,18).

In Croatia, the surveillance system of HIV and other STI is based on case reporting and successive cross-sectional studies of populations under heightened risk within the framework of second generation HIV surveillance. The aim of this study was to analyze the prevalence and risk factors for HIV infection, hepatitis B and syphilis in populations with high-risk behaviors.

PATIENTS AND METHODS

During a three-year period (2011-2013), a seroepidemiological survey of HIV, hepatitis B, and syphilis in groups with high-risk behaviors (MSM, homo/bisexual persons, CSW/clients of CSW, persons with multiple sexual partners, IDUs) was conducted in 11 Croatian counties within the framework of second generation HIV surveillance. Study participants were recruited with the help of epidemiologists and non-governmental organizations that provide services for most-at-risk populations, mostly among clients of voluntary counselling and testing centers at counties public health institutes (convenience sampling).

After obtaining informed consent for participation in the study, each participant filled an anonymous questionnaire regarding sociodemographic data and risk-behaviors, followed by venipuncture for HIV, hepatitis B, and syphilis testing. All study participants received counseling and education on HIV and other STDs (22,23).

Serologic tests were performed at the Laboratory for Serology, Croatian Institute of Public Health (CIPH) using commercial diagnostic tests: HIV p24Ag/anti-HIV1/2, HBsAg, and anti-HBc using enzyme-linked fluorescent assay (ELFA; Mini Vidas, Marcy l'Etoile, France) and syphilis using enzyme-linked immunosorbent assay (ELISA; Euroimmun, Lübeck, Germany) and western blot test (WB; Euroimmun, Lübeck, Germany) for confirmation of positive results. All HIV ELFA reactive samples were confirmed using a western blot at the University Hospital for Infectious Diseases "Dr Fran Mihaljević", Zagreb.

The study was approved by the Ethics Committee of the CIPH.

Statistical analysis

Man-Whitney U and Fisher's exact tests were used to compare differences between groups of ordinal and nominal variables, respectively. Assumption of binomial distribution was used for confidence intervals calculation. The level of statistical significance was set to $\alpha=0.05$. For statistical analysis, STATA/IC version 11.2 (StataCorp LP, USA) software was used.

RESULTS

Sociodemographic characteristics and risk behaviors of study population

The study included a total of 443 participants. There were 354 (79.9%) men and 89 (20.1%) women aged 18-62 years (Figure 1). One hundred and one participants (22.8%) were single/separated, 185 (41.7%) were unemployed, 153 (34.5%) had ever injected drugs, and 276 (62.3%) reported a history of travelling/staying abroad for extended periods. All participants reported one or more high risk sexual behaviors: 99 (22.3%) MSM / bisexual orientation, 276 (62.3%) having multiple sexual partners (more than two partners in previous 12 months), 22 (4.9%) SW, 103 (23.3%) clients of SW, and 305 (68.0%) reported a history of STD.

Seroprevalence results

The overall prevalence rates of HIV, HBsAg, anti-HBc, and syphilis antibodies were 1.4% (95% CI=0.5-2.9), 2.6% (95% CI=1.3-4.5), 12.1% (95% CI=9.1-15.5),

and 3.4% (95% CI=1.9-5.6), respectively. Seroprevalence results according to the participant's characteristics are presented in Table 1. Anti-HBc and syphilis seroprevalence differed among genders with higher prevalence among men (anti-HBc 13.8% vs. 5.7%, $P=0.043$; syphilis 4.4% vs. 0.0%; $P=0.049$). There were significant differences in hepatitis B and syphilis prevalence between age groups. Anti-HBc antibodies in-

creased progressively with age from 3.3% in persons less than 30 years old to 25.0% in persons aged 40-49 years and remained stable thereafter, while prevalence of HBsAg was highest in the 40-49-year age group (7.4%) compared with 1.3-3.7% in other age groups. Homo / bisexual persons showed higher seroprevalence of syphilis (12.2%) compared with heterosexual persons (1.2%, $P<0.001$). Participants with

Table 1. Prevalence of HIV, hepatitis B and syphilis according to participants' characteristics

| Characteristic | HIV | | HBsAg | | Anti-HBc | | Syphilis | |
|---------------------------|-----------------|-------|-----------------|-------|-----------------|--------|-----------------|--------|
| | N+/N tested (%) | p | N+/N tested (%) | p | N+/N tested (%) | p | N+/N tested (%) | p |
| Gender | | 0.605 | | 0.131 | | 0.043 | | 0.049 |
| Male | 6/349 (1.7) | | 11/339 (3.2) | | 40/340 (13.8) | | 15/343 (4.4) | |
| Female | 0/88 (0) | | 0/87 (0) | | 5/87 (5.7) | | 0/88 (0) | |
| Age (years) | | 0.827 | | 0.048 | | <0.001 | | 0.004 |
| < 30 | 3/155 (1.9) | | 2/153 (1.3) | | 5/153 (3.3) | | 1/155 (0.6) | |
| 30 - 39 | 2/181 (1.1) | | 3/180 (1.7) | | 24/181 (13.3) | | 5/178 (2.8) | |
| 40 - 49 | 1/72 (1.4) | | 5/68 (7.4) | | 17/68 (25.0) | | 6/69 (8.7) | |
| 50+ | 0/31 (0) | | 1/27 (3.7) | | 6/27 (22.2) | | 3/31 (9.7) | |
| Marital status | | 0.505 | | 0.488 | | 0.805 | | 0.283 |
| Single | 0/76 (0) | | 0/74 (0) | | 9/74 (12.2) | | 5/75 (6.7) | |
| Married | 4/209 (1.9) | | 6/203 (3.0) | | 22/204 (10.8) | | 7/207 (3.4) | |
| Steady partner | 1/119 (0.8) | | 3/117 (3.4) | | 17/117 (14.5) | | 3/117 (2.6) | |
| Divorced/widowed | 1/35 (2.9) | | 1/34 (2.9) | | 4/34 (11.8) | | 0/34 (0) | |
| Employment status | | 0.703 | | 1.000 | | 0.550 | | 0.003 |
| Employed | 3/249 (1.2) | | 6/246 (2.4) | | 28/246 (11.4) | | 12/181 (6.6) | |
| Unemployed | 3/185 (1.6) | | 5/177 (2.8) | | 24/178 (13.5) | | 3/247 (1.2) | |
| Sexual orientation | | 0.108 | | 0.703 | | 1.000 | | <0.001 |
| Heterosexual | 3/346 (0.9) | | 8/339 (2.4) | | 42/340 (12.4) | | 4/341 (1.2) | |
| Homo/bisexual | 3/91 (3.3) | | 3/87 (3.4) | | 10/87 (11.5) | | 11/90 (12.2) | |
| History of STDs | | 1.000 | | 0.002 | | <0.001 | | <0.001 |
| Yes | 3/305 (1.0) | | 6/73 (8.2) | | 24/74 (32.4) | | 9/75 (12.0) | |
| No | 1/76 (1.3) | | 3/298 (1.0) | | 19/298 (6.4) | | 5/302 (1.7) | |
| Sex work | | 1.000 | | 0.446 | | 0.168 | | 0.550 |
| Yes | 0/22 (0) | | 1/22 (4.5) | | 5/22 (22.7) | | 1/22 (4.5) | |
| No | 6/415 (1.4) | | 10/404 (2.5) | | 47/405 (11.6) | | 14/409 (3.4) | |
| Paying for sex | | 1.000 | | 1.000 | | 0.730 | | 1.000 |
| Yes | 1/103 (1.0) | | 2/101 (2.0) | | 11/101 (10.9) | | 3/101 (3.0) | |
| No | 5/334 (1.5) | | 9/325 (2.8) | | 41/326 (12.6) | | 12/330 (3.6) | |
| Number of sexual partners | | 0.133 | | 0.472 | | 0.904 | | 0.013 |
| <2 | 0/152 (0) | | 3/149 (2.0) | | 18/150 (12.0) | | 2/149 (1.3) | |
| 2-5 | 5/196 (2.6) | | 7/192 (3.6) | | 24/192 (12.5) | | 6/193 (3.1) | |
| >5 | 1/79 (1.3) | | 11/417 (2.6) | | 8/76 (10.5) | | 7/79 (8.9) | |
| Injecting drug use | | 0.670 | | 0.207 | | <0.001 | | 0.003 |
| Yes | 1/153 (0.7) | | 6/151 (4.0) | | 35/152 (23.0) | | 14/281 (5.0) | |
| No | 5/284 (1.8) | | 5/276 (1.8) | | 17/276 (6.2) | | 0/150 (0) | |
| History of travelling | | 0.092 | | 0.987 | | 0.759 | | 0.778 |
| Yes | 6/276 (2.2) | | 4/153 (2.6) | | 32/269 (11.9) | | 10/272 (3.7) | |
| No | 0/156 (0) | | 7/269 (2.6) | | 20/153 (13.1) | | 4/154 (2.6) | |

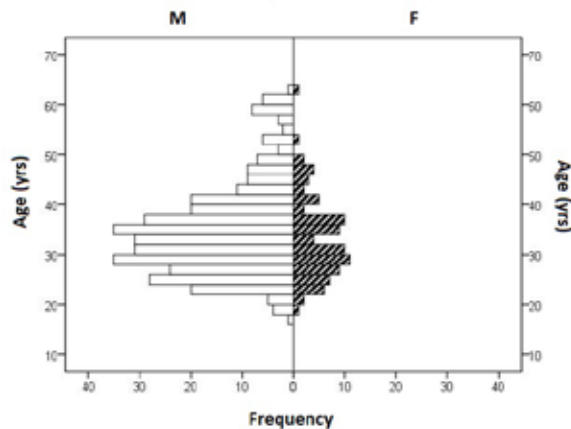


Figure 1. Distribution of study participants according to age and gender

history of STD were more often seropositive than participants who did not report STD (HBsAg 8.2% vs. 1.0%, $P=0.002$; anti-HBc 32.4% vs. 6.4%, $P<0.001$; syphilis 12.0% vs. 1.7%, $P<0.001$). In addition, syphilis seroprevalence was associated with the number of sexual partners and varied from 1.3% in persons who reported less than two sexual partners to 8.9% in persons who reported more than five sexual partners in the previous 12 months ($P=0.013$). Anti-HBc and syphilis prevalence correlated with IDU (anti-HBc 23.0% vs. 6.2%, $P<0.001$; syphilis 5.0% vs. 0.0%, $P=0.003$). Marital status, employment status, history of paid sex, and history of travelling / staying abroad for extended periods were not associated with HIV, HBsAg, anti-HBc, or syphilis seropositivity.

Analyzing sexual risk behaviors, results of the logistic regression showed that history of STD was a significant risk factor for HBsAg (OR=8.806, 95% CI=2.148-36.107; AOR=6.229, 95% CI=1.491-26.022), anti-HBc (OR=7.048, 95% CI=3.596-13.814; AOR=5.872,

95% CI=2.899-11.896), and syphilis seropositivity (OR=8.100, 95% CI=2.628-24.956; AOR=5.572, 95% CI=1.751-17.726), while homo / bisexual behavior was a significant risk factor for syphilis seropositivity (OR=11.731, 95% CI=3.639-37.808; AOR=12.820, 95% CI=3.688-44.557). In addition, syphilis seroprevalence was associated with the number of sexual partners (consecutive increase for one partner, OR=1.065, 95% CI=1.029-1.102; AOR=1.074, 95% CI=1.031-1.118). Anti-HBc seroprevalence correlated with male gender (OR=2.632, 95% CI=1.014-6.849) and IDU (OR=4.557, 95% CI=2.453-8.465; AOR=5.618, 95% CI=2.804-11.256) (Table 2 and Table 3).

DISCUSSION

In persons with high risk sexual behavior enrolled in this study, HIV prevalence was reported to be 1.4% overall, and higher among MSM (3.3%). With regard to the prevalence rates in MSM and bisexual persons with previous Croatian studies, the seroprevalence found in this study was comparable with the results of the studies conducted in 2010 and 2007 (2.8% and 3%, respectively) (23,24), while the seroprevalence was higher (4.5%) in the 2006 study (25).

The results of the SIALON Project study, conducted among MSM in six cities in Southern and Eastern Europe showed prevalence rates of 2.6% in Prague (Czech Republic), 4.6% in Bucharest (Romania), 5.1% in Ljubljana (Slovenia), 6.0% in Bratislava (Slovakia), 11.8% in Verona (Italy), and 17.0% in Barcelona (Spain) (26). Lower prevalence rates were reported in two cities in Moldova (Chisinau 2%, Balti 1%) (14,16). In this study, 0.9% heterosexual persons were found to be HIV seropositive, which is within the range of previous Croatian studies (0.6%-1.2%) (23,25). Although homo / bisexual persons showed higher seropositivity than

Table 2. Logistic regression for the risk of HIV, hepatitis B and syphilis seropositivity

| Characteristic | HIV | | HBsAg | | Anti-HBc | | Syphilis | |
|---|-----------------|----------------|-------|----------------|----------|----------------|----------|----------------|
| | OR | 95%CI | OR | 95%CI | OR | 95%CI | OR | 95%CI |
| Male vs female gender | NA ^a | NA | NA | NA | 2.632 | 1.014-6.849 | NA | NA |
| Age (one year increase) | 0.968 | 0.876 - 1.070 | 1.058 | 1.001 - 1.118 | 1.075 | 1.043 - 1.107 | 1.089 | 1.040 - 1.141 |
| Homo/bisexual vs heterosexual orientation | 3.897 | 0.773 - 19.643 | 1.478 | 0.384 - 5.691 | 0.921 | 0.442 - 1.919 | 11.731 | 3.639 - 37.808 |
| History of STDs (yes vs no) | 1.342 | 0.137 - 13.086 | 8.806 | 2.148 - 36.107 | 7.048 | 3.596 - 13.814 | 8.100 | 2.628 - 24.956 |
| Sex work (yes vs no) | NA | NA | 1.876 | 0.229 - 15.352 | 2.240 | 0.789 - 6.353 | 1.343 | 0.168 - 10.708 |
| Paying for sex (yes vs no) | 0.645 | 0.074 - 5.585 | 0.709 | 0.151 - 3.338 | 0.849 | 0.419 - 1.721 | 0.811 | 0.224 - 2.933 |
| Number of sexual partners | 1.001 | 0.909-1.003 | 0.959 | 0.814 - 1.130 | 0.972 | 0.911 - 1.037 | 1.065 | 1.029 - 1.102 |
| Injecting drug use (yes vs no) | 0.367 | 0.042 - 3.170 | 2.243 | 0.673 - 7.475 | 4.557 | 2.453 - 8.465 | NA | NA |

^aNA=not applicable

Table 3. Logistic regression for the risk of HIV, hepatitis B and syphilis seropositivity after adjusting for age and gender

| Characteristic | HIV | | HBsAg | | Anti-HBc | | Syphilis | |
|---|------------------|----------------|-------|----------------|----------|----------------|----------|----------------|
| | AOR ^a | 95%CI | AOR | 95%CI | AOR | 95%CI | AOR | 95%CI |
| Homo/bisexual vs heterosexual orientation | 3.103 | 0.609 - 15.792 | 1.353 | 0.346 - 5.299 | 0.928 | 0.430 - 1.998 | 12.820 | 3.688 - 44.557 |
| History of STDs (yes vs no) | 1.702 | 0.151 - 19.142 | 6.229 | 1.491 - 26.022 | 5.872 | 2.899 - 11.896 | 5.572 | 1.751 - 17.726 |
| Sex work (yes vs no) | NA ^b | NA | 2.212 | 0.252 - 19.430 | 2.459 | 0.791 - 7.641 | 1.449 | 0.160 - 13.105 |
| Paying for sex (yes vs no) | 0.513 | 0.058 - 4.486 | 0.389 | 0.078 - 1.943 | 0.474 | 0.216 - 1.041 | 0.368 | 0.093 - 1.453 |
| Number of sexual partners | 1.001 | 0.902 - 1.108 | 0.943 | 0.774 - 1.149 | 0.968 | 0.897 - 1.040 | 1.074 | 1.031 - 1.118 |
| Injecting drug use (yes vs no) | 0.327 | 0.037 - 2.853 | 2.059 | 0.600 - 7.067 | 5.618 | 2.804 - 11.256 | NA | NA |

^aAOR=adjusted for age and gender; NAb=not applicable

heterosexuals (3.3% vs. 0.9%), the difference was not significant. This finding might be due to the very small number of HIV seropositive subjects in our study.

Regarding hepatitis B, the overall rate of anti-HBc in this study was 12.1%, which is almost two times higher than that reported in the Croatian general population (7.0%) (27) as well as in the MSM (7.7%) (25). In groups with high risk sexual behavior within Europe, HBV prevalence showed significant geographical variations from 3.2%-9.1% in Moldova (14,16), 7% in Denmark (28), 7.6% in Albania (15), 12% in Scotland (19), to a high 27.1% in Germany (18) and 28.9% in Belgium (29). The prevalence of current HBV infection (HBsAg positive) of 2.6% found in this study is within the European range (<1-7%) (19-21,29).

Syphilis seropositivity found in Croatia during 2011-2013 was lower (3.4%) than in the 2010 (7.6%) (24) and 2006 studies (10.6%) (25), as well as in the majority of other European studies: Germany 36.9%, Italy 21.6%, Belgium 12.5%, Russia 12%, Moldova 6.1-12.1% and Albania 6.5% (14-16,18,29-31). The only exception to this trend was found in Balti, Moldova, where a very low prevalence of syphilis in MSM was found (0.5%) (14).

With respect to gender, our study showed significant differences in HBV and syphilis among genders: anti-HBc antibodies were found in 13.8% men and 5.7% women, while all participants with positive syphilis serology (3.4%) were male. A Turkish study on the prevalence of syphilis conducted among newly diagnosed HIV / AIDS patients showed similar results, i.e. found syphilis seropositivity only in men (32). However, in some countries gender differences were reported with female predominance. For example, in Moldova, seroprevalence of HBV was higher in female SW than in MSM (8.9-9.1% vs. 3.2-5.7%), while syphilis seropositivity in female SW was 6.1-8.4% compared with 0.1-12.1% in MSM (14,16). Although a difference in HIV seroprevalence between genders was also observed in this study (no women were seropositive

compared with 1.7% men), this difference was not significant, probably due to the small number of seropositive subjects.

Seropositivity to HBV and syphilis in Croatia was strongly age dependent. The possible explanations for a steady increase in prevalence of hepatitis B among older clients tested include longer lifetime exposure to HBV as well as cohort effect of the childhood vaccination program (universal infant immunization implemented in 1999 at national level) which altered hepatitis B epidemiology trends and shifted the majority of HBV infections to older age.

HIV seropositive persons were equally distributed in the age group below 50. In the SIALON Project, prevalence of HIV among people of over 25 years of age was significantly higher than among people younger than 25 years (9.6% vs. 3.5%) (26).

Regarding high risk sexual behavior, HBV positivity in this study was significantly related to a history of STD. Although a higher seroprevalence of anti-HBc was found in participants who reported sex work than in those who did not (22.7% vs. 11.6%), the difference was not significant. Probability of being syphilis seropositive was significantly higher in persons who reported homo / bisexual orientation, a history of STDs, and higher number of sexual partners.

The relationship between HIV, HBV, and syphilis with IDU among persons with high risk sexual behavior was observed in many studies (33-35). A Dutch study found HIV seropositivity to be 13.6% in IDU CSW compared with 5.7% in non-IDU CSW (35).

Lifetime IDU was common in persons tested in this study (34.5%). Participants who reported lifetime IDU were significantly more likely to be HBV and syphilis seropositive compared with those who did not (23.0% vs. 6.2% and 5.0% vs. 0%, respectively). There was no difference in HIV seropositivity among IDUs and non-IDUs (0.7% and 1.8%). In contrast, a Russian study found no association of IDU and STD / HIV among Moscow female SW (33).

Our study has some limitations. As a result of a convenience sample, inherent bias should be taken into account. Due to the small numbers of participants in some risk groups (SW) as well as a small number of HIV-positive participants, some calculations should be interpreted with caution. Despite these limitations, our results provide new data on the epidemiology, including risk factors for STD, in key population groups with different high risk behaviors in Croatia.

CONCLUSIONS

The results of this study showed that HIV seroprevalence did not change substantially, while syphilis seroprevalence decreased in Croatia over the last decade. The prevalence of hepatitis B was the highest of the STDs screened. A higher hepatitis B prevalence found in this study compared with previous Croatian studies probably reflects a high proportion of persons who reported lifetime IDU. Although prevalence of some STDs in groups with high risk sexual behavior has declined, a high proportion of sexual risk behaviors in these population groups highlight the importance of continuing STDs screening and prevention.

Conflict of interest:

The authors report no conflicts of interest regarding the manuscript.

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