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Prevalence and Risk Factors for Herpes Simplex Virus Type 2 Infections in East Croatia

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ABSTRACT

The aim of the study was to establish the seroprevalence of herpes simplex virus type 2 (HSV-2) among sexually active adults from East Croatia and to evaluate its correlates with demographic and behavioural risk factors. This cross-sectional study was conducted from the 1st June until the 30th September 2005 at the Department of Transfusion Medicine, Osijek University Hospital Center. The study included 423 blood donors composed of 366 (86.5%) men and 57 (13.5%) women. Demographic data and data on the risky sexual behaviour during the lifetime were collected with usage of an anonymous questionnaire consisting of 15 questions. Western blot testing revealed that 3.3% (14/423) subjects were positive for the presence of IgG antibodies to herpes simplex virus 2, i.e. 10 (2.7%) men and 4 (7.0%) women. A higher HSV-2 seroprevalence was associated with female sex, older age, marital status (with single subjects been under the greater risk), irregular condom use on sexual intercourses with new partners and with higher number (five or more) of sexual partners during lifetime. Among determined risk factors in the study population, only the connection between the HSV-2 positivity and the older age was statistically significant. The study has showed that the demographic rather than the behavioural risk factors are associated with higher prevalence of HSV-2 antibodies in the observed population.

Key words: *HSV-2 seroprevalence, blood donors, Western blot testing, HSV-2 antibodies, Croatia*

Introduction

Herpes simplex virus type 2 (HSV-2) infections are considered to be almost always sexually transmitted[1–3], and it has been established that the presence of antibodies to HSV-2 correlates highly with past sexual behaviour of a person and/or his or her sexual partners[4]. Exact proof for the fact that HSV-2 is almost exclusively sexually transmitted (with the exception of rare perinatal acquisition) is data from the surveys investigating the seroprevalence of HSV-2 among the subjects from different subpopulation groups. These had showed how seroprevalence of antibodies to HSV-2 in sera of the subjects that had never been sexually active is negligible or equal to zero[5].

In recent years, HSV glycoprotein (gG) was identified as a viral protein that specifies predominantly type-specific epitopes, and measurement of antibodies directed against HSV-2 glycoprotein G (gG2) is now possible by the use of commercially available glycoprotein G2-based enzyme immunoassays. The use of those has largely simplified the evaluation of seroprevalence of HSV-2 in epidemiological studies[4,6,7]. Besides that, there is also a possibility of using the Western blot assay that has more than 98% sensitivity and specificity for distinguishing between specific herpes simplex type 1 (HSV-1) and herpes simplex type 2 (HSV-2) antibodies[4,8,9]. Western blot is considered to be the traditional »gold standard« for differentiating between type 1 and type 2 antibodies[10–13], although its use is limited to only few research laboratories due to its complexity and price[12]. The most important thing is, that today, by using the combination of these two methods (type specific enzyme immunoassays and Western blot assay) it is possible to determine ones contact with HSV-2 with exact certainty.

Experts consider that almost two decades world is facing the pandemic of HSV-2 infections[4,14], with HSV-2 be-

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ing the most common cause of genital ulcers not only in the developed world but also in many developing countries as well[15–17]. Although the HSV-2 infection is one of the most common sexually transmitted diseases worldwide[18–21] and despite the fact, that according to the reports from the gynaecologists in the field who notice that occurrence of this infection is constantly increasing in Croatia[22], during the past twenty years there has not been any epidemiological report concerning the seroprevalence of HSV-2 in the Croatian population[22,23]. In the European epidemiological studies, blood donors are often used to represent the general population since all European countries have unpaid donors[24] and in performing present study we also follow such presumption. The aim of this study was to establish the seroprevalence of herpes simplex virus type 2 (HSV-2) among sexually active adults from East Croatia and to evaluate its correlates with demographic and behavioural risk factors.

Subjects and Methods

This cross-sectional study was conducted from the 1st June 1 until 30th September 2005 at the Department of Transfusion Medicine, Osijek University Hospital Center. The study was approved by the Ethics Committee for Medical Research of the Medical School Osijek and the written informed consent has been obtained from each subject.

Subjects

The study initially included 440 blood donors of both sexes (of a total of 4000 blood donors registered in East Croatia). Study subjects were randomly selected among blood donors aged 20–59. Out of 440 initially selected blood donors, 427 gave their consent to take part in the study (response rate, 97%) preceded by thorough verbal and written information on the methodology and the purpose of the study.

Besides 450-mL blood unit collected, an additional 5-mL blood sample was obtained from each blood donor for serologic testing. The donors were also asked to fill-in the anonymous questionnaire on their sexual behaviour that may have implied their HSV-2 exposure. Blood sampling for study testing was performed after blood donation, from the same site as venepuncture for blood donation to obviate unnecessary vein puncture. Upon collection, the blood samples were transported to the Department of Microbiology, Public Health Institute of the Osijek-Baranja County, where serum was prepared by the whole blood centrifugation and stored at –20°C until the analysis.

Four of 427 subjects reported that they had never been sexually active and were then excluded from the study as being incompatible with the study aim and hypothesis as well as because of literature data confirming the HSV-2 seroprevalence being negligible in individuals that have never been sexually active[11,25]. The rest of 423 subjects that reported sexual activity were included in further laboratory and statistical analysis.

Anonymous questionnaire

Study subjects were asked to fill-in an anonymous questionnaire in order to identify different demographic and behavioural risk factors that may have implied their exposure to HSV-2. There were 15 questions, 13 of them of closed type (with optional answers to be marked) and two of open type (the year of the birth and the age at the first sexual contact). Demographic data included the place of residence, age, sex, level of education, and marital status. Data on sexual preferences included questions of sexual activity during the lifetime, the age at the first sexual contact, partner sex, receiving or giving payment for sex during the lifetime, the type of sexual activities during the lifetime, the regularity of condoms usage during the sexual contacts with new partners, lifetime history of sexually transmitted diseases (STDs), number of sexual partners during the lifetime, sexual activity and the number of sexual partners in the past year. The questionnaire employed as a research tool in the present study was previously validated in a small group of blood donors in 2004. The questionnaire used in this study was presented in details in Table 1.

Laboratory analysis

The blood samples collected, i.e. whole blood sera, were analysed by use of the commercial anti-HSV-2 ELISA (IgG) test kit (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany), strictly according to the manufacturer's instructions[26]. This assay identifies IgG antibodies to G2 glycoprotein, considered as the only reliable marker of HSV-2 infection, while detection of antibodies to HSV-2 G2 glycoprotein is considered to reliably demonstrate the subject's contact with this virus type[19–21]. All ELISA positive and ELISA equivocal blood samples were additionally tested with the anti-HSV-2 gG-2 EUROLINE-WB (IgG) Western blot test kit (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany), strictly following the manufacturer's instructions[27]. Upon confirmation testing, all blood samples were classified as definitely positive or definitely negative, and then submitted to statistical processing.

Statistics

Descriptive statistics were used for data processing and analysed using SPSS Statistical Package for Windows, version 13.0 (SPSS Inc., Chicago, IL, USA). Normality of data distribution was tested with the Kolmogorov-Smirnov test; the χ^2-test and the Fisher exact test were used to determine differences in the distribution of qualitative variables. On all statistical analyses, two-sided p-values of 0.05 and lesser ones were considered significant.

Results

The final study sample of 423 sexually active donors from East Croatia consisted of 366/423 (86.5%) male and 57/423 (13.5%) female subjects, mean age 34.6±10.5

(range 20.0–57.0) years. According to the place of residence, 283/423 (66.9%) subjects were from the urban setting and 140/423 (33.1%) from the rural setting. There were 323/423 (76.4%) subjects with high school education, 68/423 (16.0%) with finished university, and 32/423 (7.6%) had lower than high school education. According to marital status, there were 233/423 (55.1%) married and 190/423 (44.9%) single subjects.

According to the sexual orientation there were 421/423 (95.5%) heterosexual subjects and 2/423 (0.5%) homosexual or bisexual subjects. Among all the study subjects there were 15/423 (3.5%) of them who reported receiving or giving payment for the sex during the lifetime. According to the practiced types of sexual intercourses there were 219/423 (51.8%) study subjects who reported practicing solely vaginal type of sexual intercourse and 204/423 (48.2%) study subjects who reported practicing different types of sexual intercourses. Irregular condom use with new sexual partners during the lifetime was reported by 204/423 (48.2%) of all the study subjects. Among all the study subjects there were 19/423 (4.5%) of them who had been diagnosed with any kind of STD at least once during the lifetime. According to their answers in anonymous questionnaires among all the study subjects there were 213/423 (50.4%) of them who had five or more sexual partners during lifetime and 106/423 (25.1%) of them who had two or more sexual partners during the one year that precede this study.

The Western blot testing of donor blood revealed 14 (3.3%) of 423 blood samples to be positive for the presence of HSV-2 IgG antibodies, including 10/366 (2.7%) male and 4/57 (7.0%) female donors. Sex difference in the positivity for HSV-2 IgG antibodies was not statistically significant (Fisher exact test=2.830; p=0.0925) (Figure 1).

Since age is an important determinant of sexual behaviour[28,29], study subjects were divided into two age groups: the younger group, aged 20–39 (n=285) and the older group, aged 40–59 (n=138) years regarding all the analysis of HSV-2 IgG findings. Findings positive for HSV-2 IgG antibodies were recorded in 6/285 (2.1%) subjects from the younger group and 8/138 (5.8%) subjects from the older group. Difference in the rate of findings positive for HSV-2 IgG antibodies between the two age groups was statistically significant (Fisher exact test =3.960; p=0.0465) (Figure 2). According to the marital status HSV-2 IgG antibody positivity was determined in 3.0% (7/233) married subjects and 3.7% (7/190) single subjects but this difference was not statistically significant (Fisher exact test=0.151; p=0.6974). Considering the prevalence of the behavioural risk factors for HSV-2 infection in HSV-2 IgG positive and negative subjects,

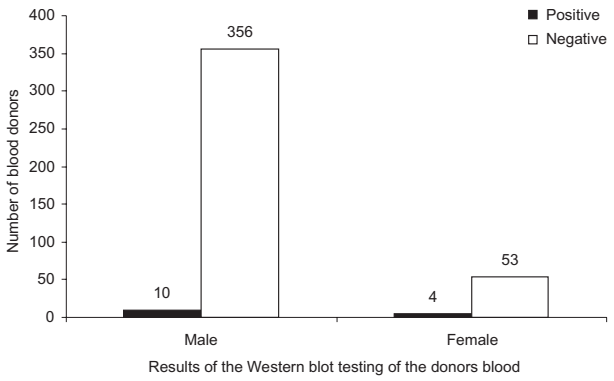

Fig. 1. Blood donors from East Croatia positive and negative for HSV-2 IgG antibodies according to the sex.

TABLE 1
ANONYMOUS QUESTIONNAIRE FOR BLOOD DONORS – STUDY SUBJECTS IN THE STUDY ABOUT THE HERPES SIMPLEX VIRUS TYPE 2

Please circle or write your answer!

1. Place of residence: a) urban setting b) rural setting
2. The year of birth:
3. Sex: Male Female
4. Level of education:
   a) unfinished elementary school
   b) finished elementary school
   c) finished high school
   d) finished college
   e) finished faculty
5. Marital status:
   a) married
   b) single (never married)
   c) divorced / widowed
6. Have you ever, during lifetime, been sexually active: YES NO
7. Age at first intercourse:
8. Sexual orientation: a) heterosexual b) homosexual c) bisexual
9. Have you ever, during the lifetime, paid for or being paid for sexual services: YES NO
10. Type of sexual intercourses that you have been practicing during the lifetime:
    a) I have never been sexually active
    b) Solely vaginal type of sexual intercourse
    c) Mostly vaginal and besides them sometimes anal and/or oral type of sexual intercourse
    d) Solely anal type of sexual intercourse
    e) Solely oral type of sexual intercourse
11. During the sexual intercourses with new partners during the lifetime I have always used condoms: YES NO
12. How often, during the lifetime, have you been diagnosed with any type of sexually transmitted disease: a) never (0 times) b) once (1 time) c) 2 or more times
13. Number of sexual partners during the lifetime:
    a) 0 b) 1 c) 2–4 d) 5–9 e) 10–19 f) 20 or more
14. Have you been sexually active during the last year: YES NO
15. Number of sexual partners during the last year:
    a) 0 b) 1 c) 2 d) 3 or more

Thank you very much for your cooperation!

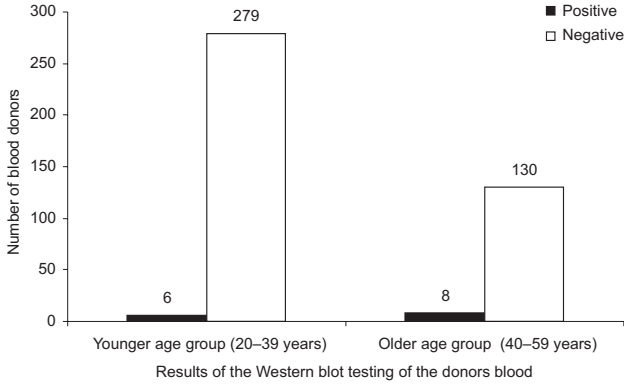

Fig. 2. Blood donors from East Croatia positive and negative for HSV-2 IgG antibodies according to the age groups.

only the irregular condom use on sexual intercourses with new partners and higher number (five or more) of sexual partners during lifetime were connected with the HSV-2 IgG antibody positivity, but none of those were statistically significant (Fisher exact test=0.562; p=0.4535 and χ^2=2.572; p=0.1088, respectively).

Among the younger and the older groups of blood donors there were differences in the prevalence of the various types of behavioural risk factors for HSV-2 infection. In the younger group of the blood donors there were 2/285 (0.7%) homosexual and bisexual oriented blood donors, while in the older group of the blood donors all of them were heterosexual. Within the younger group of the blood donors there was a larger proportion of the blood donors who received or gave payment for sex during the lifetime, who practiced different types of sexual intercourses, and who had two or more sexual partners during the year that precede this study than in the older group of the blood donors with the last two differences being statistically significant (χ^2=14.823, p<0.0001; χ^2= 5.258, p=0.0218). Within the older group of the blood donors there was a larger proportion of them who had one or more STDs during the lifetime, who had five or more sexual partners during the lifetime, and who reported the irregular condom use with new sexual partners during the lifetime than in the younger group of the blood donors with the last difference being statistically significant (χ^2=5.161, p=0.0231) (Table 2).

Discussion and Conclusion

The present study that included blood donors from East Croatia was the first study specifically investigating HSV-2 seroprevalence in the general Croatian population. Following the customary practice in majority of European epidemiological studies we presumed that the blood donors were representative for the general population[24]. Using the Western blot analysis, the presence of HSV-2 IgG antibodies was recorded in 3.3% (14/423) of donor blood samples, as a reliable indicator of their previous contact with HSV-2[19–21].

The Western blot testing of donor blood samples from other European countries (Poland, Italy, Norway, Germany, and United Kingdom) showed the seroprevalence of HSV-2 in blood donors from these countries to range from 5.0% to 11.0%. These values are comparable to Croatian samples despite the fact that seroprevalence of HSV-2 in Croatian blood donors was a little bit lower[3,4,12,30]. As the variations in HSV-2 seroprevalence resulted from different levels of HSV-2 dissemination in the populations of the respective countries[12,17], it can be concluded

TABLE 2
BEHAVIOURAL RISK FACTORS FOR HSV-2 INFECTION AMONG BLOOD DONORS FROM EAST CROATIA ACCORDING TO THE AGE GROUPS

Behavioural risk factors for HSV-2 infection		Age groups		Statistical analysis
		Younger group	Older group	
Receiving or giving payment for sex during the lifetime	Yes	13 (4.6%)	2 (1.4%)	Fisher exact test=2.633; p=0.1047
	No	272 (95.4%)	136 (98.6%)	
Types of sexual intercourses during the lifetime	Different types	156 (54.7%)	48 (34.8%)	χ^2=14.823; p<0.0001*
	Only vaginal	129 (45.3%)	90 (65.2%)	
Irregular condom use with new sexual partners during the lifetime	Yes	188 (66.0%)	106 (76.8%)	χ^2=5.161; p=0.0231*
	No	97 (34.0%)	32 (23.2%)	
Number of STDs during the lifetime	1 or more	12 (4.2%)	7 (5.1%)	Fisher exact test=0.161; p=0.6882
	0	273 (95.8%)	131 (94.9%)	
Number of sexual partners during the lifetime	5 or more	138 (48.4%)	75 (54.3%)	χ^2=1.306; p=0.2530
	1–4	147 (51.6%)	63 (45.7%)	
Number of sexual partners during the year that precede this study	2 or more	81 (28.4%)	25 (18.1%)	χ^2=5.258; p=0.0218*
	0 or 1	204 (71.6%)	113 (81.9%)	

*statistically significant difference

that the prevalence of HSV-2 in the general population of Croatia is lower in comparison with the industrialized countries of Western and Northern Europe.

In the present study, higher HSV-2 seroprevalence was associated with female sex, older age, marital status (with single subjects been under the greater risk), irregular condom usage during the sexual intercourses with new partners and with higher number (five or more) of sexual partners during lifetime and these findings are consistent with the findings of other similar surveys[31–34].

The fact that the female sex is a risk factor for HSV-2 infection is explained by the more efficient male to female transmission than female to male transmission[6,35] of this virus and by the anatomic features of the female genital[10]. Concerning this, it has been emphasized that besides the exposure of a larger genital surface in women, female mucosa and genital skin is more susceptible to herpes simplex viruses' type 1 or type 2 than the penile epithelium[10].

Among other risk factors some studies pointed to the older age as a risk factor (with persons of 35 and more years being under the greater risk)[7,32–34]. It is believed that the age is closely connected with another risk factor, the duration of sexual activity, where, obviously, larger number of years of sexual activity represents greater risk[2,34,36].

Although some studies had pointed out to the connection between lower education, early age of first sexual intercourse (younger than 17 years), homosexual and bisexual orientation, prostitution, practice of anal sexual intercourses, history of sexually transmitted diseases and larger number of sexual partners during the one year prior to the investigation of HSV-2 seroprevalence in a population and ones HSV-2 positivity[13,25,36] our study did not confirm these connections.

Comparisons between the younger and the older group of the blood donors had detected differences in proportions of various behavioural risk factors for HSV-2 infection. In that sense it is especially interesting to emphasize that although the blood donors from the younger group had more frequently involved themselves in the various types of the risky sexual behaviour they had also more frequently used condoms for protection. These data are consistent with the results reported from the other studies conducted among the Croatian youth, which had showed the rate of condom usage to be higher among the younger individuals who generally have a greater number of sexual partners[37–39]. In this way, by the frequent use of condoms as a method of protection, young people tend to neutralize the effect and prevent the occurrence of the detrimental consequences (primarily HIV infection) of their more liberal attitude towards promiscuous relations[37,40].

The first limitation of the present study is very low overall number of positive subjects. Because of that we probably failed to succeed in our attempt to confirm the association between HSV-2 positivity and lower educational status, early onset of sexual activity (before the age of 17) and practice of anal sexual intercourses that had been shown in some other studies[22]. The second limitation of this study results from the features of the sexual behaviour of blood donors. Despite the fact that blood donors are representative for the general population one can still say that this population is in fact the low risk population concerning the sexual behaviour[12,17,22] (in which some forms of risky sexual behaviour such as homosexual and/or bisexual orientation, prostitution, history of sexually transmitted diseases and large number of sexual partners during the one year prior to the enrolment in the HSV-2 seroprevalence investigation are quite rear) and because of that the present study did not show the connection between HSV-2 positivity and some of the above mentioned forms of risky sexual behaviour[22].

Finding that among blood donors there are also HSV-2 positive individuals confirms the fact that genital herpes is one of the most common sexually transmitted disease worldwide[1,5,20,21] which public health significance is constantly growing[18,29,41]. Concerning this, one must bear on mind that fight against the viral sexually transmitted diseases (such as HSV-2 infections but also HIV disease) cannot be effective if directed only towards high risk groups[22,42], but must also include preventive actions in low risk groups (such as blood donors), because only that comprehensive approach truly protects the whole population[22,43].

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PREVALENCIJA I RIZIČNI ČIMBENICI ZA NASTANAK INFEKCIJA VIRUSOM HERPES SIMPLEX TIP 2 U ISTOČNOJ HRVATSKOJ

S A Ž E T A K

Cilj ovoga istraživanja bio je utvrditi seroprevalenciju virusa herpes simplex tipa 2 (HSV-2) među spolno aktivnim odraslim osobama s područja istočne Hrvatske, te procijeniti njezinu povezanost s demografskim osobinama, te rizičnim oblicima spolnog ponašanja ispitanika. Ova *cross-sectional* studija provedena je u razdoblju od 1. lipnja do 30. rujna 2005. godine na Odjelu za transfuzijsku medicinu Kliničkog bolničkog centra Osijek. Istraživanje je uključilo 423 dobrovoljnih davatelja krvi (DDK) od kojih je bilo 366 (86,5%) muškaraca i 57 (13,5%) žena. Demografski podatci o ispitanicima, te podatci o njihovom spolno rizičnom ponašanju tijekom života dobiveni su s pomoću anonimnog anketnog upitnika koji se sastojao od 15 pitanja. Western blot testiranjem krvi DDK na prisustvo protutijela klase IgG na herpes simplex virus tipa 2 utvrđeno je kako su uzorci krvi 14 (3,3%) dobrovoljnih davatelja bili pozitivni na prisutnost protutijela klase IgG na herpes simplex virus tipa 2, pri čemu je pozitivnost utvrđena u 10 (2,7%) muškaraca te 4 (7,0%) žene. Viša seroprevalencija HSV-2 bila je povezana sa ženskim spolom, starijom dobi, bračnim statusom (pri čemu su samci imali veći rizik), neredovitom uporabom kondoma s novim spolnim partnerima tijekom života, te s većim brojem (pet ili više) spolnih partnera tijekom života. Među utvrđenim rizičnim čimbenicima za nastanak infekcije virusom HSV-2 u promatranoj populaciji jedino je povezanost između starije dobi ispitanika te pozitivnosti na HSV-2 bila statistički značajna. U promatranoj populaciji, demografske osobine ispitanika su značajnije povezane s višom prevalencijom HSV-2 nego li njihovo rizično spolno ponašanje.