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HIGH-RISK HUMAN PAPILLOMAVIRUS (HPV) INFECTION IN WOMEN UNDERGOING IN VITRO FERTILIZATION

OKUŽBE Z VISOKORIZIČNIMI GENOTIPI HUMANIH PAPILOMSKIH VIRUSOV (HPV) PRI ŽENSKAH, VKLJUČENIH V POSTOPKE ZUNAJTELESNE OPLODITVE

Nina Jančar[1], Eda Vrtačnik Bokal[1], Mario Poljak[2], Anja Oštrbenk[2]

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Abstract

Objective: *The aim of our study was to establish the prevalence of high-risk human papillomavirus (hr-HPV) infection in a population of women included in an in vitro fertilisation (IVF) program and to correlate the outcome of IVF cycles with HPV status.*

Methods: *A total of 195 women undergoing the IVF program were included in the study. A cervical smear for cytological analysis and hr-HPV determination was collected from every woman. RealTime High Risk HPV test (Abbott Molecular Inc., Des Plaines, IL) has been used for the detection of hr-HPV infection. All participants were invited to complete an anonymous questionnaire that included questions regarding medical and sexual history as well as risk factors for HPV infection. HPV 16 and HPV 18 positive women were invited for follow-up gynaecological examinations, including colposcopy 4 to 6 months after the inclusion.*

Results: *Mean age of included infertile women was 33.7 ± 4.36 years. A total of 16/195 women (8.2%) were hr-HPV positive. Hr-HPV infection was not associated with the percentage of mature oocytes, the percentage of fertilised oocytes, with embryo quality or with pregnancy rate in our study.*

Conclusions: *The prevalence of hr-HPV genotypes in Slovenian infertile women undergoing IVF is lower than the hr-HPV prevalence in the general population. We were unable to find an association between hr-HPV cervical infection and the outcome of IVF cycles.*

Key words: human papillomavirus, in vitro fertilisation, pregnancy rate

Izvirni znanstveni članek
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Izvleček

Izhodišča: *Namen naše raziskave je bil ugotoviti zastopanost okužbe z visokorizičnimi genotipi humanih papilomskih virusov (vr-HPV) v populaciji žensk, vključenih v postopke zunajtelesne oploditve (ZTO). Nadalje smo želeli ugotoviti, ali ima okužba s HPV vpliv na izid postopkov ZTO.*

Metode: *Vključili smo 195 žensk, ki so se zdravile s postopki ZTO. Vsaki ženski smo odvzeli bris maternične vratu za citološko preiskavo in bris za določanje okužbe z vr-HPV. Za dokazovanje okužbe z vr-HPV smo uporabili test RealTime High Risk HPV (Abbott Molecular Inc., Des Plaines, IL). Vsako žensko smo prosili, da izpolni anonimni vprašalnik, ki je vključeval ginekološko anamnezo in dejavnike tveganja za okužbo s HPV. Ženske, ki so bile HPV 16 ali HPV 18 pozitivne, smo povabili na kontrolni pregled in kolposkopijo po štirih do šestih mesecih.*

Rezultati: *Povprečna starost vključenih neplodnih žensk je bila 33,7 ± 4,36 leta. V naši raziskavi je bilo 16/195 žensk (8,2 %) vr-HPV pozitivnih. Okužba z vr-HPV ni imela vpliva na število ali odstotek zrelih jajčnih celic, odstotek oplojenih jajčnih celic, kakovost zarodka ali na stopnjo zanositve.*

Zaključki: *V populaciji žensk, vključenih v postopke ZTO, je prevalenca okužbe z vr-HPV nižja kot v splošni populaciji slovenskih žensk. Okužba z vr-HPV ni bila povezana z uspehom postopkov ZTO.*

Ključne besede: humani papilomski virusi, zunajtelesna oploditev, stopnja zanositve

[1]Department of Obstetrics and Gynecology, University Medical Centre Ljubljana, Šlajmerjeva 3, 1000 Ljubljana, Slovenia
[2]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Zaloška cesta 4, 1000 Ljubljana, Slovenia
Correspondence to: e-mail: ninajancar@hotmail.com

1 INTRODUCTION

Human papillomaviruses (HPV) are a large and heterogeneous group of viruses etiologically linked to several benign and malignant neoplasms of the skin and mucous epithelia. To date, more than 100 HPV genotypes are known and for twelve of them (HPV 16, HPV 18, HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58 and HPV 59) there is sufficient evidence that they cause cervical cancer. Those genotypes are therefore known as high-risk HPV (hr-HPV) genotypes (1). In a recent study (2), it has been found that HPV 16, HPV 18 and HPV 33 are the most common hr-HPV genotypes in women with cervical cancer in Slovenia. Several studies have shown that hr-HPV testing increases the sensitivity of detecting cervical intraepithelial neoplasia grade 2 (CIN 2) or worse in conjunction with cytology (3, 4). Consequently, hr-HPV testing has become an important part of cervical cancer screening and cervical precancerous lesions management algorithms in the last decade (5 – 8).

HPV infection is a very common sexually transmitted infection (STI). It is well known that STI like *C.trachomatis* and *N.gonorrhoea* have an important impact on fertility in women as well as men (9), but little is known about the connection between HPV infection and infertility. HPV have been found in semen, but the association of HPV infection with sperm parameters has not been resolved (10). No correlation has been found between cervical HPV infection and the cause of female infertility (11). Furthermore, no association has been found between HPV detection in cervical smear and the outcome of in vitro fertilisation (IVF) treatment (12, 13). In a small North American study (14), however, the authors have found that cervical HPV infection is associated with decreased pregnancy rate after IVF.

The risk of acquiring HPV infection increases with the increasing number of sexual partners (15). It is assumed that infertile patients included in the IVF program are in long-term monogamous relationships. Thus, it could be hypothesised that those women have lower hr-HPV infection prevalence than women of similar age groups in the general population.

The primary aim of our study was to establish the prevalence of hr-HPV genotypes in a population of women included in an IVF program. Additionally, we wanted to explore the association of cervical hr-HPV infection and the outcome of IVF cycles.

2 MATERIALS AND METHODS

All women who started hormonal treatment for IVF at the Department of Obstetrics and Gynecology, University Medical Centre Ljubljana, Slovenia, from January to May 2010 were included in the study. Controlled ovarian hyperstimulation was achieved with recombinant gonadotrophins and GnRH agonists or antagonists. Fertilisation was attempted by classical IVF or intracytoplasmic sperm injection (ICSI), where appropriate. Embryos were cultured to the blastocyst stage. One or two embryos were transferred on day 5 after oocyte retrieval. The study design was approved by the National Medical Ethics Committee (Consent Number 83/11/09) and written informed consent was obtained from all participating women. All participants were invited to complete an anonymous questionnaire that included questions regarding medical and sexual history as well as risk factors for HPV infection. Data about gynaecological history, history of STI, usage of oral contraception, previous HPV testing and HPV vaccination were also collected by the gynaecologist performing initial examination.

A cervical smear for cytological analysis was collected from every woman. A cytological slide was prepared in the conventional manner and examined by a board-certified cytopathologist. Another cervical smear was obtained using a Cervex-Brush (Rovers Medical Devices, Oss, The Netherlands) and stored in ThinPrep PreservCyt Solution (Hologic, Marlborough, MA).

A total of 20µl of ThinPrep PreservCyt Solution was used for testing with RealTime High Risk HPV test (Abbott Molecular Inc., Des Plaines, IL). RealTime assay was performed on the fully automated nucleic acid preparation instrument *m*2000*sp* and the real-time PCR instrument*m*2000rt (Abbott), following the manufacturer's instructions, as previously described (16, 17). The assay uses four channels for the detection of fluorescent probes; one for detection of an internal process control for sample adequacy, DNA extraction and amplification (136-bp region of human beta-globin), a second for detection of HPV16, a third for detection of HPV18 and a fourth for the aggregate detection of 12 HPV genotypes: HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66 and HPV68 using modified GP5+/6+ primer mix consisting of three forward and two reverse primers (16). The assay's cutoff is set up at a fixed cycle threshold value of 32 cycles.

HPV 16 and HPV 18 positive women were invited for follow-up gynaecological examinations including

colposcopy 4 to 6 months after the inclusion. Biopsy of any suspicious lesion was performed and the specimens were evaluated by a board-certified pathologist.

Statistical analysis was performed with Statistical Package for Social Sciences (SPSS) 19.0 for Windows (SPSS, Inc., Chicago, IL, USA). Kolmogorov-Smirnov test was applied to test for a normal distribution of all variables. Chi-square test, Mann-Whitney test, variance analysis, logistic regression and multinomial logistic regression were used when appropriate. Differences were considered statistically significant when *P* values were < 0.05.

3 RESULTS

A total of 195 women were included in the study. Mean age was 33.7 ± 4.36 years (range 20 to 42 years). Mean number of retrieved oocytes was 7.7 ± 5.7, mean percentage of mature oocytes was 71.7 ± 28.15% and mean percentage of fertilised oocytes was 53.0 ± 29.48% per woman. Mean number of embryos was 4.0 ± 3.6 per woman; mean number of blastocysts was 1.1 ± 1.4 per woman. A total of 157/195 women (80.5%) had embryo transfer. A total of 56/195 women were pregnant, the pregnancy rate per cycle thus being 28.7%. A total of 56/157 women with embryo transfer were pregnant, the pregnancy rate per embryo transfer thus being 35.7%.

Mean age at first sexual intercourse was 18.1 ± 2.81 years (range 13 to 37 years). Most included women were non-smokers (N=150; 76.9%). A total of 187/195 women (95.9%) had normal cytological smear; 6 women (3.1%) had reactive changes in the cytological smear, one woman (0.5%) had atypical squamous cells and one woman (0.5%) had mild dyskaryosis.

A total of 179/195 women (91.8%) were hr-HPV negative and 16/195 women (8.2%) were hr-HPV positive; 3 women (1.5%) were infected with HPV 16, 2 women (1.0%) were infected with HPV 18 and 11 women (5.6%) were infected with other hr-HPV genotypes. Only one out of 6 women (16.7%) with reactive cytological changes was HPV positive. The only woman with atypical squamous cells was HPV negative, and the only one with mild dyskaryosis was hr-HPV positive.

Data about previous gynaecological history and STI were missing in 14/195 women (7.2%). A total of 161/181 women (89.0%) had no history of vulvovaginal disease or STI; 20/181 women (11.0%) had history of fungal or bacterial vulvovaginal infection or STI such as genital herpes, *C.trachomatis*, genital warts or pelvic inflammatory disease.

Hr-HPV positive women had more sexual partners in their lifetime than hr-HPV negative women (3.7 ± 3.24 versus 7.1 ± 7.2 partners; P = 0.006). Only one woman (0.5%) who was hr-HPV negative had two partners in the last year.

The percentage of pregnant women was lower in the group of women who smoked compared to those who were non-smokers (15.6% vs. 32.7%; P = 0.026).

The differences between hr-HPV positive women and hr-HPV negative women in mean age, percentage of mature and fertilised oocytes, development of blastocysts on day 5 and pregnancy rate per cycle are shown in table 1. Hr-HPV infection was not associated with the percentage of mature oocytes. Hr-HPV infection had no effect on the percentage of fertilised oocytes, embryo quality or pregnancy rate. Only advancing female age was associated with less oocytes retrieved, less embryos and less blastocysts (P < 0.001, P = 0.002, P = 0.002, respectively). The history of other STI had no effect on HPV status.

Gynaecological exam with colposcopy was performed in HPV 16 and HPV 18 positive women 4-6 months after inclusion. A total of 4/5 women (80%) had normal colposcopic appearance of the uterine cervix. One HPV 16/18 positive woman with cytologically diagnosed mild dyskaryosis had a suspicious lesion, which was biopsied. Histological diagnosis was cervical intraepithelial neoplasia grade 1 (CIN 1).

Table 1. Mean age and the parameters of IVF success in hr-HPV negative and hr-HPV positive women.
Tabela 1. Povprečna starost in parametri uspešnosti postopkov ZTO pri VR-HPV negativnih in VR-HPV pozitivnih ženskah.

Parameter	HR-HPV negative (N = 179) / VR-HPV negativne	HR-HPV positive (N = 16) / VR-HPV pozitivne	P value / P vrednost
Mean age / Povprečna starost	33.9 ± 4.31 years	31.9 ± 4.28 years	NS*
Percentage of mature oocytes / Odstotek zrelih jajčnih celic	71.6 ± 28.3%	70.1 ± 31.7%	NS*
Percentage of fertilised oocytes / Odstotek oplojenih jajčnih celic	52.9 ± 29.7%	50.8 ± 30.4%	NS*
Percentage of blastocysts on day 5 / Odstotek blastocist 5. dan	57.0%	75.0%	NS#
Pregnancy rate per IVF cycle / Stopnja zanositve na postopek ZTO	29.6%	18.8%	NS#

*Mann-Whitney test
#Chi-Square test

4 DISCUSSION

A total of 8.2% of women included in the IVF program were hr-HPV positive, which is less than the mean prevalence of hr-HPV in women aged 30-35 years in Central Europe (≈12%) (18). Lundquist et al. (11) also found that HPV infection is somewhat less prevalent in women included in an IVF program than in the general population in Sweden (7% versus 9%). The overall prevalence of hr-HPV in the recent Slovenian HPV Prevalence Study was 12.9% (19). The prevalence of hr-HPV positive women in our study was lower than that established in the Slovenian HPV Prevalence Study performed on 4,432 women consecutively enrolled in 16 outpatient gynaecology services (a convenience sample with national coverage) (19). The mean age of women in the two study groups was however quite similar (33.7 in our study and 36.6 in Slovenian HPV Prevalence Study). We speculate that this difference could be due to the different sexual behaviour of the general population of women versus infertile women. Namely, the majority (99.5%) of infertile women treated with assisted reproduction techniques in our study had only one sexual partner in the last year.

The only statistically significant difference between hr-HPV positive and hr-HPV negative women was the mean number of lifetime sexual partners. Hr-HPV positive women had significantly more lifetime sexual partners than hr-HPV negative women, and this fact additionally supports the above hypothesis.

The overall incidence of cervical cytological abnormalities was also low in our study. Every woman included in IVF in Slovenia is supposed to have a PAP smear taken three or less years prior to inclusion in an IVF program. Based on cytological result, the woman should receive treatment if necessary prior to inclusion in an IVF program.

Infection with hr-HPV genotypes was not associated with the number of retrieved oocytes, the percentage of mature or fertilised oocytes or with embryo quality or pregnancy rate. Such results were expected, since HPV infects mainly squamous epithelial cells, and therefore does not interfere with the parameters of IVF success. The major drawback of our study was the relatively low number of included women. Nevertheless, our results were similar to those obtained by Wang et al. (12) on 1,044 Chinese women undergoing IVF. They also found that IVF outcome was not associated with HPV infection, cervical inflammation or cervical precancerous lesions.

5 CONCLUSION

In conclusion, the prevalence of hr-HPV genotypes in Slovenian infertile women undergoing IVF is lower than the hr-HPV prevalence in the general population of Slovenian women. High-risk HPV infection was not associated with the outcome of IVF cycles in our study.

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References

1. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F et al. A review of human carcinogens - Part B: biological agents. Lancet Oncol 2009; 10: 321-322.
2. Jancar N, Kocjan BJ, Poljak M, Lunar MM, Bokal EV. Distribution of human papillomavirus genotypes in women with cervical cancer in Slovenia. Eur J Obstet Gynecol Reprod Biol 2009; 145: 184-188.
3. Lytwyn A, Sellors JW, Mahony JB, Daya D, Chapman W, Howard M, et al. Adjunctive human papillomavirus testing in the 2-year followup of women with low-grade cervical cytologic abnormalities. Arch Pathol Lab Med 2003; 127: 1169–1175.
4. Arbyn M, Paraskevaidis E, Martin-Hirsch P, Prendiville W, Dillner J. Clinical utility of HPV-DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN: an update of pooled evidence. Gynecol Oncol 2005; 99: S7–S11.
5. Wright TC Jr, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D. 2006 American Society for Colposcopy and Cervical Pathology-sponsored Consensus Conference. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. Am J Obstet Gynecol 2007; 197: 346-355.
6. Arbyn M, Anttila A, Jordan J, Ronco G, Schenck U, Segnan N, et al. European guidelines for cervical quality assurance in cervical cancer screening: summary document. 2nd ed. Ann Oncol 2010; 21: 448-458.
7. Cox JT. History of the use of HPV testing in cervical screening and in the management of abnormal cervical screening results. J Clin Virol 2009; 45: S3-S12.
8. Uršič-Vrščaj M, Rakar S, Možina A, Kobal B, Takač I, Deisinger D, et al. Smernice za celostno obravnavo žensk s predrakavimi spremembami maternične vratu. Ljubljana: Onkološki inštitut, 2011.
9. Pellati D, Mylonakis I, Bertoloni G, Fiore C, Andrisani A, Ambrosini G, et al. Genital tract infections and infertility. Eur J Obstet Gynecol Reprod Biol 2008; 140: 3-11.
10. Ochsendorf FR. Sexually transmitted infections: impact on male fertility. Andrologia 2008; 40: 72-75.
11. Lundqvist M, Westin C, Lundkvist Ö, Simberg N, Strand A, Andersson S, et al. Cytologic screening and human papillomavirus test in women undergoing artificial fertilization. Acta Obstet Gynecol Scand 2002; 81: 949–953.
12. Wang Y, Wang C, Qiao J, Wang L, Liang S. Relationship of cytopathology and cervical infection to outcome of in-vitro fertilization embryo transfer. Int J Gynecol Obstet 2008; 101: 21-26.
13. Tanaka H, Karube A, Kodama H, Fukuda J, Tanaka T. Mass screening for human papillomavirus type 16 infection in infertile couples. J Reprod Med 2000; 45: 907-911.
14. Spandorfer SD, Bongiovanni AM, Fasioulotis S, Rosenwaks Z, Ledger WJ, Witkin SS. Prevalence o cervical human papillomavirus in women undergoing in vitro fertilization and association with outcome. Fertil Steril 2006; 86: 765-767.
15. Almonte M, Silva Idos S, Asare A, Gilham C, Sargent A, Bailey A, et al. Sexual behavior and HPV infection in British women, by postal questionnaires and telephone interviews. J Med Virol 2011; 83: 1238-1246.
16. Huang S, Tang N, Mak WB, Erickson B, Salituro J, Li Y, et al. Principles and analytical performance of Abbott RealTime HR HPV test. J Clin Virol 2009; 45: S13–S17.
17. Poljak M, Ostrbenk A, Seme K, Ucakar V, Hillemanns P, Bokal EV, et al. Comparison of clinical and analytical performance of the Abbott Realtime High Risk HPV test to the performance of hybrid capture 2 in population-based cervical cancer screening. J Clin Microbiol 2011; 49: 1721-1729.
18. De Vuyst H, Clifford G, Li N, Franceschi S. HPV infection in Europe. Eur J Cancer 2009; 45: 2632-2639.
19. Učakar V, Poljak M, Klavs I. Pre-vaccination prevalence and distribution of high-risk human papillomavirus (HPV) types in Slovenian women: a cervical cancer screening based study. Vaccine 2012; 30: 116-120.