Original Article

Comparison of the prevalence of human papillomaviruses among fertile and infertile women in Mashhad, northeast of Iran

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Abstract

Background: Human papillomaviruses (HPVs) are the most common viruses which can be sexually transmitted. They can cause different malignancies in asymptomatic women. The association of HPVs with infertility among men and women is controversial. In the current study, the authors compared the frequency of HPVs in fertile and infertile women in the city of Mashhad.

Materials and Methods: In the present case-control study, cervical and vaginal smears were collected from infertile and fertile women. HPVs were detected by polymerase chain reaction. Data was analyzed by SPSS v.20 and P-value <0.05 was considered statistically significant.

Results: In the current study, 115 infertile women with the mean age of 30.5±5.6 years and 60 fertile women with the mean age of 32.6±9.3 years were included (p=0.07). Among women who were infertile (cases), 121 (52.6%) of 230 smears were positive, while in control group (who were fertile), 50 (41.7%) of 120 smears were positive (p=0.052).

Conclusion: Frequency of HPV in both groups was high, which could be due to lack of routine HPV vaccination. HPV can cause placenta abnormality, our infertile women had multiple abortion history and history of abortion had significant differences among infertile and control group. The frequency of HPV had no significant differences between the infertile and control groups.

Keywords: Human papillomavirus, infertility, polymerase chain reaction

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Introduction

Failure to become pregnant after one year of regular unprotected sexual intercourse is defined as infertility. Infertility is one of the most common problems among young couples. It is estimated that in industrialized nations, 15% of people are infertile (1). Therefore, it has become a top priority for many health organizations and governments to tackle this issue. The reasons responsible for infertility are categorized in three different groups: genetic, anatomic and environmental factors. Infection is one of the environmental reasons which can cause infertility among men and women. Few studies suggested HPV as one of the causes of infertility in women (2).

Human papillomaviruses (HPVs) are non-enveloped viruses with circular double stranded DNA (3, 4). They are the most common viruses which can be sexually transmitted. They can be transmitted through skin contact or any other surface contact related to the genital area. HPVs are distributed in the skin and mucous membranes, including the pubic area, oral cavity and perianal region. The lifetime risk of being infected with HPVs is 80% for any woman in the world (2). The age at which people begin sexual activities, being an immune compromised patients and the number of sexual partners, are positively related with this infection. About 100 different HPV types are known that are able to cause a variety of complications including malignancy due to the infection of basal keratinocytes of the epidermis (5, 6). The most common types which cause cervical cancers are types 16 and 18 (7-11). Screening test is recommended for all sexually active women, since most of the infected women with type 16 and 18 have no symptoms until they develop malignancies. Polymerase chain reaction test is one of the accurate ways for detecting HPVs (12). The aim of the current study was to compare the frequency of HPVs in infertile and fertile women, effects of marriage duration and HPV frequency, compare HPV frequency in cervical and vaginal specimens and relation between history of abortion and infertility in Mashhad, northeast of Iran.

Methods

The current case control study was performed from 2012 to 2015. Approval was received from the Research Ethics Committee of Mashhad University of Medical Sciences. The current study was supported by Mashhad University of Medical Sciences, Mashhad, Iran. (Grant No. 900506). Informed written consent was obtained from 115 infertile women who were admitted to the center for reproduction at Montaserieh hospital in Mashhad and 60 fertile women as a control group. All participants had no history of smoking, addiction, high risk sexual behavior and other medical complications such as autoimmune diseases. All participants had one sexual partner. Cervical and vaginal smears were taken by gynecologists.

DNA was extracted using extraction buffer consisted of Tris-Cl (100 mM, pH 7.5) and Tween-20 (0.05%) as described previously (13). Prepared samples were used directly for polymerase chain reaction (PCR) amplification without more purification steps. To assess the quality of extracted DNA, PCR of β-globin gene was performed.

In the current study, the quality of the extracted DNA was determined by amplification of a 260-bp fragment of the β-globin gene. PC04 and GH20 primers were used for PCR amplification. PCR reaction mixture contained 1 μL (100 ng) of DNA, 0.8 μL of Taq DNA polymerase (5 U/μL, CinnaGen, Iran), 0.4 μL of dNTPs (10 mM), 1.6 μL of MgCl2 (25 mM), 2.5 μL of 10X PCR buffer, and 10 pmol/μL of each primer, in a total reaction volume of 25 μL. PCR amplification was performed for 35 cycles (94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s) after an initial denaturation step of 94 °C for 5 min. The cycles were followed by a 7-min extension at 72 °C. PCR product was visualized on a 2% agarose gel by green viewer (Pars Tous, Iran) staining and UV photography.

In order to determine the presence of HPVs in the extracted DNA samples, GP5+ (5´-TTTGTTACTGTGGTAGATACTAC-3´) and GP6+ (5´-GAAAAATAAACTGTAAATCATATTC-3´) primers were used to amplify a 142-bp fragment of L1 which was a highly conserved sequence of HPV genome. By performing this PCR, almost all HPV types, including high and low risk types, could have been identified. PCR reaction mixture consisted of 1 μL of DNA (100 ng/μL), 0.3 μL of Taq DNA polymerase (5 U/μL, CinnaGen, Iran), 0.4 μL of dNTP (10 mmol), 0.6 μL of MgCl2 (50 mmol), and 10 pmol/μL of each primer, in a total volume of 25 μL. PCR program was as follows: denaturation for 5 min at 94 °C; 35 cycles of 94 °C for 45 s, 47.5 °C for 45 s, and 72 °C for 45 s; and a final extension of 72 °C for 7 min. Fragment sizes of PCR products were assessed by comparison with a 100 bp DNA size marker in a 2% agarose gel.

Data was analyzed by SPSS v.18 (Chicago, IL, USA) software and Chi-Square test, Mann-Whitney U test, Fisher's Exact Test were used. For control confounding variables, Logistic regression was applied to compare between the duration of marriage (years), the history of abortion, and the HPV infection with the

chance of infertility. A p-value <0.05 was considered statistically significant.

Results

All samples showed a band corresponding to 260-bp in the PCR of β-globin gene. Samples were amplified for a fragment of a highly conserved region of L1 gene of HPV (HPV L1). Among the infertile women, 121 (52.6%) of 230 smears were positive, while 50 (41.7%) of 120 smears were positive in the control group (p=0.052) (Figure 1).

Age distribution in infertile and fertile women is shown in figure 2. The mean of age of infertile women was 30.5±5.6 years and the mean of age of fertile women was 32.6±9.3 years (p=0.07). HPV infection was detected in infertile and fertile women by PCR method and the differences was not statistically significant (P-Value =0.052).

The presence of HPV infection (evaluated by PCR) among the infertile and fertile women according to their age is shown in Table 1.

The history of previous abortion was found in 15 (60%) and 19 (26.4%) of infertile and fertile women, respectively. The differences between infertile and fertile groups in history of previous
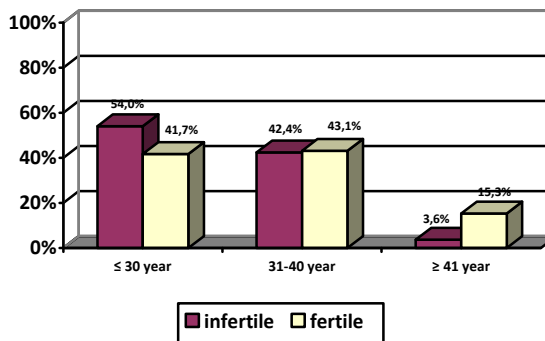

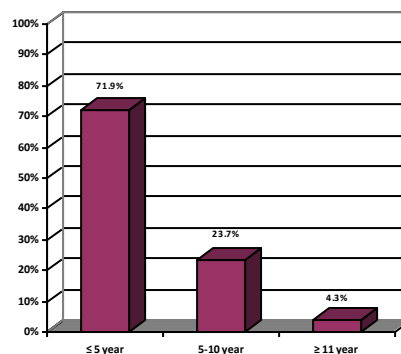**Figure 2.** Age distribution in infertile and fertile women

Figure 3. The distribution of the duration of infertility in the infertile women

abortion was statistically significant (p=0.002). The number of abortions in infertile and fertile women was analyzed based on the number of abortions and it was shown to be statistically significant (P-Value=0.0001) (Table 2). The status of HPV infection was evaluated among the infertile and fertile women based on their previous abortions and the differences was not statistically significant.

The mean of the duration of marriage in infertile group was 6.1±3.4 years while in control group, it was 9.6±7.2 years. The status of HPV infection (evaluated by PCR) in infertile and fertile women according to the duration of marriage was evaluated and the difference was not statistically significant. The distribution of the duration of infertility in the infertile group is shown in figure 3.

Vaginal and cervical specimens were used in our study and the DNA extraction was performed by the same method in both specimens. HPV infection was detected in the extracted DNA by PCR. The status
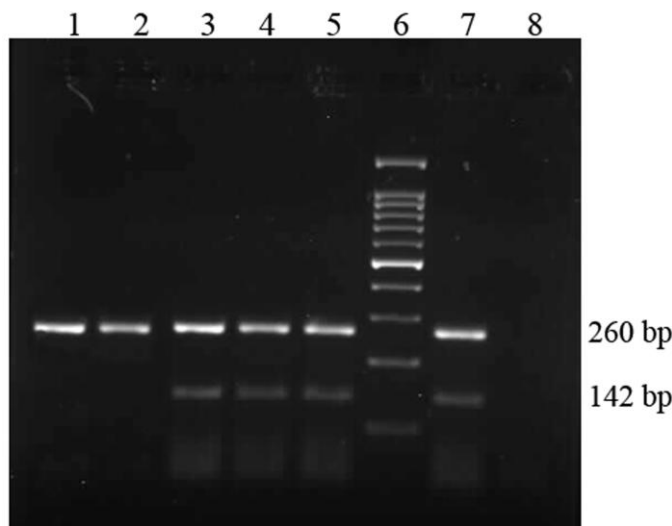
Figure 1. Agarose gel electrophoresis of β-globin and HPV L1 PCR products. PCR was used for amplification of a 260bp fragment of β-globin and a 142bp fragment of HPV L1 fragments. Lanes 3, 4, and 5 represent β-globin and HPV L1 positive samples. Lanes 7 and 8 are negative and positive controls for the PCR amplification, respectively. Lanes 1 and 2 showed HPV L1 negative samples. Lane 6 represents the 100 bp DNA size marker.

Table 1: Distribution of HPV infection (evaluated by PCR) among the infertile and fertile women according to their age

Age (year)	Study groups	HPV infection Positive (*n* (%))	HPV infection Negative (*n* (%))	Total (*n* (%))	Statistic Test *P-Value*
≤ 30	Infertile	66(55.0%)	54(45.0%)	120(100%)	5.24[#]
	Fertile	20(36.4%)	35(63.6%)	55(100%)	0.022*
31-40	Infertile	49(49.0%)	51(51.0%)	100(100%)	0.120[#]
	Fertile	26(52.0%)	24(48.0%)	50(100%)	0.729
≥41	Infertile	6(60.0%)	4(40.0%)	10(100%)	[$]
	Fertile	4(26.7%)	11(73.3%)	15(100%)	0.122

Pearson Chi-Square
$Fisher's Exact Test
* Statistically significant

Table 2: The number of abortions among infertile and fertile women

No. of abortions	Infertile (*n* (%))	Fertile (*n* (%))	Total (*n* (%))	Statistic Test* *P*-Value
0	9 (36.0%)	53 (73.6%)	62 (63.9%)	
1	9 (36.0%)	15 (20.8%)	24 (24.7%)	-3.62
2	5 (20.0%)	3 (4.2%)	8 (8.2%)	0.0001**
3	2 (8.0%)	1 (1.4%)	3 (3.1%)	
Total (*n* (%))	25 (100%)	72 (100%)	97 (100%)	

* Mann-Whitney U
** Statistically significant

Table 3: Distribution of HPV infection in infertile and fertile according to type of specimens using PCR

type of specimens	Study groups	HPV infection Positive (*n* (%))	HPV infection Negative (*n* (%))	Total (*n* (%))	Statistic Test* *P*-Value
Vaginal specimens	Infertile	54(43.5%)	70(56.5%)	124(100%)	0.231
	Fertile	28(40.0%)	42(60.0%)	70(100%)	0.631
Cervical specimens	Infertile	67(63.2%)	39(36.8%)	106(100%)	5.11
	Fertile	22(44.0%)	28(56.0%)	50(100%)	0.024**

* Pearson Chi-Square
** Statistically significant

of HPV infection based on type of specimens were evaluated and it was shown to be statistically different in infertile and fertile women (P-Value=0.024) (Table 3).

No association was found between the duration of marriage and the positive HPV test (p=0.08).

Logistic regression analysis between the duration of marriage (years), the history of abortion, and HPV infection with a chance of infertility showed history of abortion increased chance of infertility by about 3.68 times that is statistically significant while positive HPV infection that increases the chance of infertility by 1.43%, is not statistically significant (Table 4).

Discussion

HPV is one of the most common sexually transmitted diseases that affects between 100 and 200 new cases per 100,000 adults in the general population in the world per year (14, 15). A recent study on 825 married women aged 18-59 years from the general population of Tehran (Iran) showed that the prevalence of HPV was 7.8%, with no significant

Table 4: Logistic regression between the duration of marriage (years), the history of abortion, and the HPV infection with the chance of infertility

Variable(s) entered on step 1	Sig.	Exp(B)
Duration of marriage	0.583	1.019
History of abortion	0.001**	3.681
HPV positive results	0.353	1.430

* Logistic regression
** Statistically significant

variation by age (16). In the present study, the prevalence of HPV was 48.6%. These differences may have been due to the geographical differences or the relatively lower number of participants in the current study. HPV positivity is more common among young males and females, due to have a higher tendency to find new partners at the peak age of new partner acquisition (17).

In the present study, 52.6% of infertile women were positive for HPVs. However, in Lundqvist et al study, 7% of them were positive (18). This difference may have been due to vaccination to protect young women against HPV types 6, 11, 16 and 18 (19). Health authorities in Iran are strongly recommended to provide nation-wide vaccination against HPV among young girls. In Spandorfer et al study in New York (USA), HPV was detected in 17 (16.0%) of 106 infertile patients. They showed that HPV-positive women had a decreased pregnancy rate (4 of 17, 23.5%) as compared with HPV-negative women (51 of 89, 57.0%; P<.02) (20). In Coso's study, 75% of HPV infected infertile women became fertile after receiving treatment for HPV (21). In a recent study by Perino et al, it was showed that HPV infected couples had an increased risk of pregnancy loss after assisted reproduction technique compared with non-infected couples (22).

The role of HPV in causing infertility in women and men is controversial. In some studies, it was showed that HPV caused infertility in men by changing the motility and morphology of sperms (23, 24). However, Schillaci and colleagues found that only 24 of 308 semen samples (7.8%) were HPV DNA positive in infertile men (25). HPV cervical infection has risk factors for tub peritoneal sterility,

and patients are also in risk of other sexually transmitted diseases. In the current study, similar to that of Lundqvist's study (in which the frequency of HPV in infertile and fertile women were 7.0% and 9.1%, respectively), there was no significant differences between the frequency of HPV in infertile and fertile groups (18). Also, Strehler and colleagues found no statistically significant differences in the prevalence of HPV DNA between infertile women and healthy control women (26). In other studies in different countries, HPV had a higher prevalence rate among infertile group compared to that of control group (20, 27, 28).

In Ambuhl study, they found significant difference between HPV prevalence and spontaneous abortion (29). According to some studies HPV considered as one of the reason of abortion by causing abnormal implantation and placental dysfunction in pregnant women (30, 31). In this study, there was significant difference between history of abortion and infertility. Our infertile women had multiple abortion history. To our knowledge, in the worldwide, there was no previous study regarding the association of the duration of marriage in HPV affected patients. The authors found no relationship between the duration of marriage with being infected with HPV.

Conclusion

In conclusion, in the current study, the frequency of HPV had no statistically significant association among infertile and fertile women. However, the prevalence of HPV in both groups of participants was high. Thus, it is not possible to exclude the possible adverse effects of HPV infection on infertility. In other words, its early detection may lower the frequency of cervical cancer and infertility.

Conflicts of Interest

The authors declared that they had no financial interests related to the material in the manuscript.

Acknowledgment

References

1. Homan G, Davies M, Norman R. The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: a review. Human Reproduction Update. 2007;13(3):209-23.

2. Koutsky L. Epidemiology of genital human papillomavirus infection. The American Journal of Medicine. 1997;102(5):3-8.

3. Nayereh KG, Khadem G. Preventive and therapeutic vaccines against human papillomaviruses associated cervical cancers. Iranian Journal of Basic Medical Sciences. 2012;15(1):585.

4. Meshkat Z, Soleimanjahi H, Mahmoudi M, Mirshahabi H, Hassan ZM, Ghaffari SR, et al. Determination of human papillomavirus type 16 genotype and construction of cloning vector pTZ57R encoding HPV16 E7 gene. Saudi Medical Journal. 2007;28(10):1511-5.

5. Mirshahabi H, Soleimanjahi H, Pourpak Z, Meshkat Z, Hassan ZM. Production of Human Papilloma Virus Type 16 E6 Oncoprotein as a Recombinant Protein in Eukaryotic Cells. Iranian Journal of Cancer Prevention. 2012;5(1):16-20.

6. Faridi R, Zahra A, Khan K, Idrees M. Oncogenic potential of Human Papillomavirus (HPV) and its relation with cervical cancer. Virology Journal. 2011;8(1):1.

7. Meshkat Z, Hassan Z, Soleimanjahi H, Mahmoodi H, Mirshahabi H, Langeroudi MF. Detection of human papilloma viruses type 16 and 18 by PCR and RFLP in paraffin-embedded cervical cancer tissue specimens. Archives of Razi Institute. 2006;61(3):159-65, 87.

8. Meshkat Z, Soleimanjahi H, Mahmoudi M, Hassan ZM, Mirshahabi H, Meshkat M, et al. CTL responses to a DNA vaccine encoding E7 gene of human papillomavirus type 16 from an Iranian isolate. Iran J Immunol. 2008;5(2):82-91.

9. Meshkat Z, Soleimanjahi H, Mirshahabi H, Meshkat M, Kheirandish M, Hassan ZM. Strong immune responses induced by a DNA vaccine containing HPV16 truncated E7 C-terminal linked to HSP70 gene. Iran J Immunol. 2011;8(2):65-75.

10. Mirshahabi H, Meshkat Z, Soleimanjahi H, Bamdad T, Hassan Z. Isolation of Iranian human papillomavirus type 16 E6 gene and construction of its cloning vector. Pakistan Journal of Biological Sciences. 2006;9(14):2652-6.

11. Meshkat Z, Mirshahabi H, Soleimanjahi H, Mohamad Hassan Z. Construction a DNA Vaccine Containing Human Papillomavirus Type 16 Early Genes as a Potential Vaccine for Cervical Cancer Prevention and Therapy. Iranian Journal of Pathology. 2009;4(2):65-70.

12. Shen-Gunther J, Yu X. HPV molecular assays: defining analytical and clinical performance characteristics for cervical cytology specimens. Gynecologic oncology. 2011;123(2):263-71.

13. Mirshahabi H, Meshkat Z, Soleimanjahi H, Hassan ZM, Meshkat M. Different DNA extraction methods for paraffin-embedded pathological samples. Iran J Pathol. 2007;2(4):159-64.

14. Gross G, Pfister H. Role of human papillomavirus in penile cancer, penile intraepithelial squamous cell neoplasias and in genital warts. Medical Microbiology and Immunology. 2004;193(1):35-44.

15. Patel H, Wagner M, Singhal P, Kothari S. Systematic review of the incidence and prevalence of genital warts. BMC infectious diseases. 2013;13(1):1.

16. Khodakarami N, Clifford GM, Yavari P, Farzaneh F, Salehpour S, Broutet N, et al. Human papillomavirus infection in women with and without cervical cancer in Tehran, Iran. International Journal of Cancer. 2012;131(2):E156-E61.

17. Wellings K, Collumbien M, Slaymaker E, Singh S, Hodges Z, Patel D, et al. Sexual behaviour in context: a global perspective. The Lancet. 2006;368(9548):1706-28.

18. Lundqvist M, Westin C, Lundkvist Ö, Simberg N, Strand A, Andersson S, et al. Cytologic screening and human papilloma virus test in women undergoing artificial fertilization. Acta Obstetricia et Gynecologica Scandinavica. 2002;81(10):949-53.

19. Forinash AB, Yancey AM, Pitlick JM, Myles TD. Safety of the HPV bivalent and quadrivalent vaccines during pregnancy. Annals of Pharmacotherapy. 2011;45(2):258-62.

20. Spandorfer SD, Bongiovanni AM, Fasioulotis S, Rosenwaks Z, Ledger WJ, Witkin SS. Prevalence of cervical human papillomavirus in women undergoing in vitro fertilization and association with outcome. Fertility and sterility. 2006;86(3):765-7.

21. Alvarez FJ, Bustos LH, Villanueva DC, Zambrana CM, Coria SI, Aranda FC. Epidemiologic characteristics in women with sterility and cervical lesions with and without human papilloma virus infection. Comparative study. Ginecologia y Obstetricia de Mexico. 1998;66:157-63.

22. Perino A, Giovannelli L, Schillaci R, Ruvolo G, Fiorentino FP, Alimondi P, et al. Human papillomavirus infection in couples undergoing in vitro fertilization procedures: impact on reproductive outcomes. Fertility and sterility. 2011;95(5):1845-8.

23. Yang Y, Jia C-W, Ma Y-M, Zhou L-Y, Wang S-Y. Correlation between HPV sperm infection and male infertility. Asian J Androl. 2013;15(4):529-32.

24. Garolla A, Lenzi A, Palù G, Pizzol D, Bertoldo A, De Toni L, et al. Human papillomavirus sperm infection and assisted reproduction: a dangerous hazard with a possible safe solution. Human Reproduction. 2012;27(4):967-73.

25. Schillaci R, Capra G, Bellavia C, Ruvolo G, Scazzone C, Venezia R, et al. Detection of oncogenic human papillomavirus genotypes on spermatozoa from male partners of infertile couples. Fertility and sterility. 2013;100(5):1236-40.

26. Strehler E, Sterzik K, Malthaner D, Hoyer H, Nindl I, Schneider A. Influence of ovarian stimulation on the detection of human papillomavirus DNA in cervical scrapes obtained from patients undergoing assisted reproductive techniques. Fertility and sterility. 1999;71(5):815-20.

27. Repper CF, Carranza-Lira S, Alcivia GJ, Gaona AR, García LA. [Modified factors in the study of the infertile couple in women with papilloma virus cervical infection, descriptive analysis]. Ginecologia y obstetricia de Mexico. 1995;63:502-4.

28. Zhang L, Zhang H, Pei J, He G, Sun X, Li B. [Investigation on HPV viral load and high risk HPV types infection among patients with infertility]. Chinese Journal of Experimental and Clinical Virology. 2007;21(2):159-61.

29. Ambühl LM, Baandrup U, Dybkær K, Blaakær J, Uldbjerg N, Sørensen S. Human Papillomavirus Infection as a Possible Cause of Spontaneous Abortion and Spontaneous Preterm Delivery. Infect Dis Obstet Gynecol. 2016; 2016: 3086036.

30. Gomez L. M., Ma Y., Ho C., McGrath C. M., Nelson D. B.,

Parry S. Placental infection with human papillomavirus is associated with spontaneous preterm delivery. Human Reproduction. 2008; 23(3):709–715

31.   Boulenouar S, Weyn C, Van Noppen M, Moussa Ali M,

Favre M, Delvenne PO, et al. Effects of HPV-16 E5, E6 and E7 proteins on survival, adhesion, migration and invasion of trophoblastic cells. Carcinogenesis. 2010; 31(3):473–480.