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The prevalence of Human Papilloma Virus (HPV) infection in the oligospermic and azoospermic men

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Abstract

Background: Human papilloma virus (HPV) infection is one of the most common sexually transmitted diseases that affects men like women and infected cutaneous and mucosal squamous epithelium. The aim of the present study was to determine the prevalence of HPV in the semen of oligospermic, azoospermic and normal patients.

Methods: From June 2012 to June 2013, a total of 90 individuals were enrolled in this cross sectional comparative study. The participants were classified into three groups (oligospermia, azoosprmia and normal). This classification was based on a new WHO reference values for human semen characteristics published on 2010. After extraction of DNA from specimens L1 gene of HPV was amplified by nested polymerase chain reaction (Nested-PCR) and the PCR products of positive specimens were genotyped using INNO-LiPA HPV Genotyping Extra assay.

Results: Among 50 confirmed oligospermic male, 15 were HPV DNA positive (30%). In azoospemic group we had 8 HPV DNA positive (40%) and in normal group just 3 of 20(15%) samples were positive. Statistical assessment was done with SPSS v.15. Chi-square test showed no significant relationship between 3 groups results. Based on independent samples t-test, we found statistical significant relationship for sperm count ($p<0.05$) and sperm motility (slow) ($p<0.05$) in oligospermic group positive samples compared with negative. In the present study, 13 HPV genotypes were detected among positive samples. HPV genotypes 16, 45 in the high risk group and 6,11,42 in the low risk group were more frequent than the others.

Conclusion: The current study shows that HPV infection can affect on sperm count and motility and decrease count of sperm cell and decrease motility capability of these cells.

Keywords: Papilloma virus, Male infertility, Oligospermia.

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Introduction

Human papilloma virus (HPV), A group of small non-enveloped, double-stranded DNA viruses and one of the most common sexually transmitted agent, that implicate males and females from various regions (1).These viruses have worldwide distribution with over 120 known genotype and can be identified based on their tropism, one that infects skin and cutaneous surfaces (μ, β, γ, ν subtypes) and one that infects internal, moist-squamous mucous membranes (α) (2). Pubic area, oral cavity, perianal and genital tract are regions that can be infected

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(3,4), These viruses can be transmitted through skin contact or any other surface contact, it may be horizontal (by genital-genital, manual-genital, or oral-genital contact) or vertical (from mother to fetus) (2,5).

More than 90% of all cancers of the cervix caused by high-risk HPVs (oncogenic: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82; probably carcinogenic: 26, 53, 66) (6) (HPV-16 more than 50% and HPV-18 about 20%); the low-risk HPVs, such as 6,11,40, 42, 43, 44, 54, 61, 70, 72, 81, are generally associated with genital warts; the cutaneous HPV-2 causes common warts, especially in children (7,8). HPV infection is very common both among men and women. Around 75% of sexually active people will have an HPV infection at some point of life (8-12). Most HPV infections among males are benign (e.g., genital warts) and often infrequent and assessments shows that have no tendency for malignancy too (8,13,14). Several new studies shows viral infections of genital tract may play important role for male infertility (15), deterioration of spermatogenesis (15), can affect on sperm quality and parameters (16) and impairment of sperm function such as count or motility (17-19)

HPV DNA and RNA have been well documented in anal region, perineal area, scrotum, glans, penile shaft, urethra and reproductive system (testis, epididymis, and ducts deferens) (16,18, 20-22). Several studies have documented the presence of HPV in the semen with different prevalence (12,23).

Hence, the aim of the current study was to investigate the status of HPV infection in Iranian males by detection of HPV in the semen of oligospermic, azoospermic and normal patients that confirmed with semen analysis, and assessment the effects of HPV infection on semen parameters and characteristics of HPV genotype distribution to promote further discussion about the effects of HPV infection on male infertility.

Methods

Patients

The study protocol was approved by the local ethics committee. From June 2012 until June 2013 we accumulate ejaculated samples among males aged 26-55 years (median 39 years) attending to fertility clinics.

By Computer-Aided semen Analysis (CASA) system and based on new WHO reference values for human semen characteristics published on 2010 (24) we considered 3 study groups including oligospermic male group with 50 samples, azoospermic male group with 20 samples and normal male group with 20 samples.

Samples

Semen samples were collected in a private room and maintained at room temperature until complete seminal liquefaction (25). After liquefaction, semen parameters contain of sample volume, sperm concentration, sperm count, motility, and progressivity were determined using Computer-Aided semen Analysis(CASA) system (26). Approximately 1 mL of each specimen was taken for HPV DNA extraction. All extracted samples were stored in -20˚C until use (27).

DNA Extraction

After collection each semen sample was centrifuged at 2,500 rpm for 10 minutes. The supernatant was removed and the pellet was transferred to an Eppendorf tube. DNA extraction was done using a high pure viral

Table 1. MY09/11, GP05/06 primer sequence

MY09/11 forward primer (First round)	MY09/11 reverse primer
5'-CGTCCMARRGGAWACTGATC-3'	5'-GCMCAGGGWCATAAYAATGG-3'
GP05 forward primer (Second round)	GP06 reverse primer
5'-TTTGTTACTGTGGTAGATAC-3'	5'-ACTAAATGTCAAATAAAAAG-3'

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nucleic acid kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions.

Genotyping

The INNO-LiPA HPV Genotyping Extra assay (Innogenetics, Gent, Belgium) utilizes a biotinylated consensus primers (SPF10) to amplify a 65-bp region within the L1 ORF of multiple HPV types and based on reverse line blot hybridization. A poly(dT) tail was enzymatically added to the 3′ end of each of 25 oligonucleotides specific for 25 different types, namely, types 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74. Amplification of the human major histocompatibility complex, class II gene was used to monitor the sample quality and the efficiency of the DNA extraction equal volumes (10 µl each) of the biotinylated PCR products and denaturation solution (400 mmol/liter NaOH, 10 mmol/liter EDTA) were mixed in test troughs and incubated at room temperature for 5 min, after which 1 ml of prewarmed (37°C) hybridization solution (3× SSC [1× SSC is 0.15 mol/liter NaCl plus 0.015 mol/liter sodium citrate], 0.1% sodium dodecyl sulfate) was added, followed by the addition of one strip per trough. Hybridization was performed for 1 h at 50 ± 0.5°C in a closed water bath with back-and-forth shaking. The strips were then washed twice with 1 ml of washing solution (3× SSC, 0.1% sodium dodecyl sulfate) at room temperature for 20 s and once at 50°C for 30 min. Following this stringent washing, strips were rinsed twice with 1 ml of a standard rinse solution. Strips were then incubated on a rotating platform with an alkaline phosphatase-labeled streptavidin conjugate diluted in a standard conjugate solution, at 20 to 25°C for 30 min, after which strips were washed twice with 1 ml of rinse solution and once with standard substrate buffer; color development was initiated by the addition of 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium to 1 ml of substrate buffer. After 30 min of incubation at room temperature, the color reaction was stopped by aspiration of the substrate buffer and addition of distilled water. After drying, the strips were visually interpreted using a grid.

Results

Nested-PCR were done for 90 DNA extracted semen samples as described above and after that with 2% agarose gel electrophoresis, we consider 151bp region as positive (Fig. 1).

Ninety men attended to this study, the average age of them was 39.70 years (range: 22–55 years). By CASA and based on WHO guidelines, we classified these subjects to 3 groups. Of 50 subjects in the oligospermic group, 15 ones (30%) were

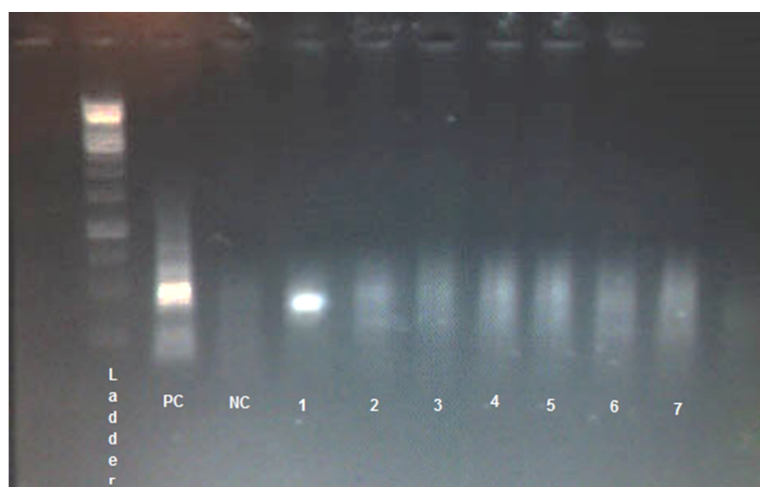

Fig.1. left to right: Ladder (100bp), positive control, negative control, between 100bp and 200bp region show as positive, another sample is negative.

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Table 2. The frequency distribution of Semen parameter

	Oligospermia	Azoospermia	Normal	Total
Age (years)	26-55	29-55	22-52	22-55
Total	50 (55.5)	20 (22.2)	20 (22.2)	90
Negative	35 (54.7)	12 (18.7)	17 (26.6)	64
Positive	15 (57.7)	8 (30.7)	3 (11.5)	26

Table 3. Descriptive statistics of Nested-PCR Result and Semen parameter

		Oligospermia (Mean±SD)	p	Azoospermia	Normal (Mean±SD)	p
Count (Million/ml)	P	7.833±3.682	<0.001	0	88.000±29.484	0.766
	N	13.745±4.463	<0.001	0	98.647±13.986	0.769
Quick progressive	P	2.200±3.569	0.057	0	23.666±7.310	0.355
Motility (%)	N	5.000±6.512	0.125	0	14.941±1.491	0.065
Slow progressive	P	29.133±8.253	0.005	0	34.666±2.728	0.337
Motility (%)	N	21.000±9.616	0.006	0	38.117±1.322	0.319
Non progressive	P	9.133±5.514	0.665	0	8.000±0.154	0.095
Motile (%)	N	8.400±5.180	0.655	0	11.000±0.813	0.156
Normal	P	6.000±2.618	0.782	0	17.333±5.333	0.831
Morphology (%)	N	6.228±2.734	0.785	0	18.647±1.191	0.710

HPV-positive, in azoospermic group with 20 subjects, 8 subjects (40%) were positive, and in normal group 3 (15%) of 20 subjects were positive (Table 2).

Chi-square test showed that the infection rate in the 3 groups was not significant between groups ($p>0.05$). Statistical analysis of semen parameters were done by independent sample t-test. In Oligospermic group, subsequently sperm count ($p<0.001$) and slow progressive motility ($p=0.005$) showed statistical significant relation between HPV positive and negative samples ($p<0.05$) (Table 3).

As well, other semen parameters were analyzed and showed no statistical significant relation (Table 3). In azoospermic group (no spermatozoa can be detected in semen sample) (32,33) we had not semen parameters (Table 3). No significant difference of sperm parameters was found between subjects of normal group (Table 2).

Analysis of HPV Genotyping

Of 28 genotypes fixed on INNO-LiPA HPV genotyping extra assay we found 13 genotypes from 26 nested-PCR positive samples. HPV genotypes 16,18,45,52 in high risk group and 6,11,34,40,42,43, 44,54,66 in low risk group were detected. In oligospermic group HPV genotypes 6,11,16,34,40,42,43,44,45,52,54,66; in azoospermic group HPV genotypes 6,11, 16,18,34,40,42,45 and in normal group just 6, 11,43 were found. HPV genotypes 6, 11, 42 among 26 HPV DNA positive subjects were more frequent than others (Table 4).

Table 4. HPV Genotyping analysis

HPV genotype	Oligospermia	Azoospermia	Normal	Total
	Highrisk			
16	1	1	0	2
18	0	1	0	1
45	1	1	0	2
52	1	0	0	1
	Low risk			
6	2	1	1	4
11	1	1	1	3
34	1	1	0	2
40	1	1	0	2
42	3	1	0	4
43	0	0	1	1
44	1	0	0	1
54	2	0	0	2
66	1	0	0	1
Total	15	8	3	26

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Discussion

The real prevalence of subclinical HPV infection in the general population and especially among men is unknown yet, because of the lack of a 'gold standard' diagnostic test for detecting subclinical HPV. The prevalence vary depends on the specific definition used, the characteristics of the populations screened, and the frequency and types of diagnostic tests employed.

Several epidemiologic studies about HPV infection in females have been reported, but data on HPV prevalence in male, especially in Iranian males is rare. In this study, 90 semen samples were tested for HPV DNA with nested-PCR that 28.88% of them had HPV infection. These samples categorized by CASA in three groups. By analyzing semen parameters and comparing with HPV positive result, we found that HPV infection can decrease the semen quality, particularly sperm count and sperm motility. Our research data suggest that HPV infected semen sample have lower sperm count and higher slow progressive motility sperm rate, which shows that HPV infection affects male fertility; therefore, HPV infection can cause infertility. Some studies show that HPV infection diminishes sperm vitality (2) and HPV virus may act on with the same mechanism to impact sperm capability like other viruses that can do it. Rintala et al found this virus in Vasa Deferens and seminal plasma sample (34). This virus localizes at the equatorial region of sperm head through interaction between the HPV capsid protein L1 and syndecan-1(2) and can decrease function of the acrosome, thus affecting the fusion of gametes. (3). HPV were found in testicular biopsies of azoospermic men and when present inside sperm cells, they may be related to impaired sperm motility and azoospermia (16). Sperm motility parameters seem to be affected by the presence of HPV in the sperm cells, and also the incidence of azoospermia may be associated with HPV infection (35). In the present study HPV-positive subjects were stable in semen parameters expect of count and motility; that they have statistical significant relationship with HPV infection in oligospermic subjects, this parameter is important and directly can lead to infertility. In oligospermic group 30% and in azoospermic group 40% were HPV positive that compared with normal group with 15% had more frequency in these groups. The most common HPV genotypes among the 90 subjects were HPV- genotypes 6, 11, 42. Further studies needs to increase numbers of samples and time to follow-up subjects and their partners.

Conclusion

This study indicated the prevalence of HPV infection and distribution of HPV genotypes among Iranian men. HPV infection may play important role in decrease of sperm cell efficiency, and frequently associated with reduced sperm motility and count and may be an important agent for oligospermia and azoospermia; that subsequently can lead to decreased male fertility or even infertility.

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