Association of Human Papillomavirus Infections with Male Infertility in Diyala-Iraq

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Abstract:

Background: Estimates suggest that worldwide between 3-7% of all heterosexual couples or women have an unresolved problem of infertility. 20-30% of infertility cases are due to male infertility**.** The prevalence of human papilloma virus (HPV) sperm infection ranges between 2 and 31% in men from general population and 10 - 35.7% in men with unexplained infertility.

Objectives: This study was designed to figure out the association of HPV type 18, 16, 11, and 6 in men with infertility in Diyala province-Iraq.

Subjects and methods: Seminal fluid samples were collected from 90 men who were subdivided equally into oligospermia, azoospermia, and healthy individuals. The age range was 22-48 years with a mean age ± SD was 29.28± 5.78 years. Socio-demographic information were also collected by direct short interview. Complete seminal fluid analysis was done according to the World Health Organization (WHO) guidelines. HPV type 18, 16, 11, and 6 were detected quantitatively and qualitatively in semen samples using the real time PCR kit (AmpliSens® *HPV* 16/18-FRT). Human privacy was respected by taken participant's consent. Statistical analyses were done using the IBM Statistical Package of Social Science (SPSS), Version 21 in association with Microsoft Excel 2010. P values less than 0.05 were considered significant.

Results: The infection rate of HPV type 18, 16, 11, and 6 were 1.1%, 6.7%, 10.0% and 1.1% respectively. The mean titer of HPV type 16 and type 18 were 188.2 and 3.0 copies/milliliter of seminal fluid respectively. The highest infection rate was in oligospermia (30%) followed by azoospermia (20%) and the viral infections were mainly affects the 26-32 years age group. HPV type 11 was significantly impaired the sperm activity (P= 0.009) and HPV type 16, 11, and 6 were significantly affects the sperm morphology (P= 0.004, 0.001, and 0.04) respectively.

Conclusion: Although HPV infections are relatively low among men with infertility; however, its effects on sperm parameters are worthy to suggest viral screening of men, at least, those couples with unexplained infertility.

Keywords: Human papilloma virus, infertility, semen quality, Diyala

Introduction:

Human papillomavirus is a small double-stranded circular DNA virus with a genome of approximately 8000 base pairs, belong to *papillomaviridae* family of which over 170 types are known, more than 40 types are transmitted through sexual contact [1]. HPVs are transmitted vertically and horizontally, and all ages can acquire both high-risk and low-risk infections through various non-sexual transmissions especially at the time of birth and by close contact even though HPV is mainly transmitted sexually [2]. Most HPV infections are clinically asymptomatic; however, it may persists and results in warts or precancerous lesions, which consequently increase the risk of cancer of the cervix, vulva, vagina, penis, anus, mouth, or throat [3]. Nearly all cervical cancer is due to HPV with two types, HPV16 and HPV18, accounting for 70% of cases, beside that between 60% and 90% of the other cancers are also linked to HPV. HPV6 and HPV11 are common causes of genital warts and laryngeal papillomatosis [4,5]. Risk factors for persistent HPV infections include early age of first sexual intercourse, multiple partners, smoking, and poor immune function [6]. Estimates of HPV prevalence vary from 14% to more than 90%, depending on the HPV strains and the target population [7].

Human papillomavirus has been detected in semen from symptomatic and asymptomatic men with testicular, accessory gland and urethral infections and associated with poor sperm quality [8]. Several studies had demonstrated that HPV sperm infection can be long lasting and frequently associated with ASAs that may further reduce male fertility [9]. Therefore, it was recommended that HPV detection and genotyping could be of great value in infertility diagnosis at least in idiopathic infertility cases [10]. A systematic review of the literature found that the prevalence of HPV sperm infection ranges between 2- 31% in men from general population and 10-35.7% in men affected by unexplained infertility. The presence of HPV in semen is associated with an impairment of sperm motility and the presence of anti-sperm antibodies [11]. In other meta-analyses, HPV DNA prevalence in semen samples varied from 0 to 100%. The pooled prevalence in infertile populations was 16% and that HPV-16 was the most common type overall [12]. An interesting finding was comes from Garolla and co-workers who reported that a reduction in natural and assisted cumulative pregnancy rate and an increase in miscarriage rate are related to the presence of HPV at sperm level [13].

Subjects and methods:

Seminal fluid samples were collected from 90 men who were subdivided equally into normal healthy individuals with normal sperm count (Over 15 million sperm per milliliter), those with oligospermia (lower than 15 million sperm /milliliter), and those with azoospermia (zero sperm of any kind found) following the definition of WHO [14]. The age range was 22-48 years. A pre-constructed questionnaire form was prepared for this purpose and filled from the participants by direct short interview. It includes information regarding age, occupation, duration of infertility, health status, history of smoking, and history of past surgical intervention. Complete seminal fluid analysis was done for participants according to the World Health Organization (WHO) guidelines for semen analysis

[15]. The HPV type 6, 11, 16 and18 were detected quantitatively and qualitatively in seminal samples using the real time PCR kit (AmpliSens® *HPV* 16/18-FRT). Human privacy was respected by taken participant's consent; however, 6 were refused enrollment. Statistical analyses were done using the IBM Statistical Package of Social Science (SPSS), Version 21 in association with Microsoft Excel 2010. P values less than 0.05 were considered significant.

Results:

Results are based on statistical analysis of data accumulated throughout this study. 90 males were included; the mean age ± SD was 29.28± 5.78 years, while the age range was 22-48 years. Regarding the infection rates, 6 (6.7%) males were positive for HPV-16, whereas 84 (93.3%) were negative. Whereas, only 1 male was positive for HPV-18 with a positivity rate 1.1%, and 89 (98.9%) were negative. Furthermore, 9 (10.0%) were positive for HPV-11. Lastly, only 1 male was positive for HPV-6 with a positivity rate of (1.1%) and 89 (98.9%) were negative.

A total of 2 (2.2%) participants were positive for viral infection in the age group < 26 years, one with HPV-16 and the another one with HPV-11. In the age group 26-32 years, 8 (8.9%) participants were positive for viral infection, 3 with HPV-16, and 5 with HPV-11. Finally, 7 (7.8%) participants were positive for viral infection in the age group > 32 years, 2 with HPV-16, 1 with HPV-18, 3 with HPV-11, and 1 with HPV-6. The table (1) showed that there was no significant association between viral infection and age groups.

Results in table (2) showed that 9 (30%), 6 (20%), and 2 (6.7%) participants with oligospermia, azoospermia, and normal health had viral infection respectively. The difference was statistically insignificant in any of HPV infection (P= 0.58, 0.36, 0.22, and 0.36) respectively.

Results in table (3) found that 4 (4.4%) of participants were positive for HPV type 16, 3 (75%) of them were with < 10 % sperm activity; with no significant association with sperm activity (P= 0.21). On the other hand, 6 (6.67%) of participants were positive for HPV type 11, and again 3 (50%) of them were with < 10% sperm activity; so, the difference was statistically significant (P= 0.009). Moreover, 1 (1.1%) was positive for HPV type 6 and it has no effect on the sperm activity (P=1.88).

Table (1): Different HPV infection rate by age groups.

Age (Ys)	HPV-16		HPV-18		HPV-11		HPV-6	
	Yes	No	Yes	No	Yes	No	Yes	No
<26	1	28	0	29	1	28	0	29
26-32	3	33	0	36	5	31	0	36
>32	2	23	1	24	3	22	1	24
χ (p value)	0.71 (0.69)		2.62 (0.26)		2.09 (0.35)		2.62 (0.26)	

Table (2): Association of HPV infection and type of subjects.

Type of subject	*HPV-16*		*HPV-18*		*HPV-11*		*HPV-6*	
	Yes	*No*	*Yes*	*No*	*Yes*	*No*	*Yes*	*No*
Oligospermia	*3*	*27*	*0*	*30*	*5*	*25*	*1*	*29*
Azospermia	*2*	*28*	*1*	*29*	*3*	*27*	*0*	*30*
Normal	*1*	*29*	*0*	*30*	*1*	*29*	*0*	*30*
χ (p value)	*1.07 (0.58)*		*2.02(0.36)*		*2.96 (0.22)*		*2.02 (0.36)*	

Table (3): Effect of HPV positivity rate titer on sperm activity.

Sperm activity	HPV-16		HPV-11		HPV-6	
	Yes	No	Yes	No	Yes	No
>10 (Active motile forward movement)	1	34	1	34	0	35
<10 (sluggish irregular movement)	3	18	3	18	1	20
Dead	0	4	2	2	0	4
χ (p value)	3.06 (0.21)		9.52(0.009)		1.88 (0.38)	

Table (4): Effect of HPV infection on sperm morphology

Viral infection	HPV-16		HPV-11		HPV-6	
	Yes	No	Yes	No	Yes	No
Normal	1	45	1	45	0	46
Abnormal	3	11	5	9	1	13
χ (P value)	8.10(0.004)		16.71(0.001)		4.06(0.04)	

Regarding HPV type -16 infection, results in table (4) revealed that 3 out of 4 (75%) of those infected participants had abnormal sperm morphology, whereas, 11 out of 56 (19.6%) of non-infected participants had abnormal sperm morphology. The difference was statistically significant (P= 0.004). In HPV type 11infection, 5 out of 6 (83.3%) of those infected participants had abnormal sperm morphology versus 9 out of 54 (16.7%) of those non-infected had abnormal sperm morphology, and again there was significant effect of HPV-type 11 infection on sperm morphology (P=0.001). Lastly, the only one participant (100%) positive for HPV type 6 had abnormal morphology, while 13 out of 59 (22.03%) of non-infected participants had abnormal sperm morphology, and hence there was statistically significant effect of HPV- type 6 infection on sperm morphology (P= 0.04). Therefore, collectively all types of viral infection except HPV type 18 had significant effect on sperm morphology.

Discussion:

Epidemiological data indicate that infertility is a problem of global concern, affecting one- fifth of couples trying to conceive worldwide and according to the trends observed, the problem is predicted to increase by another two million cases annually affecting men and women equally [16,17]. It has been estimated that infertility affects more than 10% of the world's population and in developing countries there where severe social, psychological and economic consequences for infertile men and women [18,19]. Undoubtedly, infections in the urogenital tract were accepted causes of male infertility [20,21]. Of these, infection with HPV has gained its maximum worldwide concern after definite proofs of being responsible for the development of almost all cervical cancers, 85% of anal cancer and 50% of penile, vulvar, and vaginal cancers, plus increasingly found in a subset of head and neck cancers [5]. Numerous studies on genital HPV infection in women have been conducted, but data on HPV prevalence and type distribution among Iraqi's men are scarce. Therefore, this study was arranged to explore the association between HPV infection and male infertility in Diyala province. Probably the most important limitation in this study was the small sample size included and this is mainly because that this study is personally funded and the costs of imported laboratory diagnostic kits are too expensive.

Regarding the infection rate, the present results showed that 17 out of 90 (18.8%) seminal fluids were positive for HPV infection, and that the HPV-type11 was found to be the most prevalent viral type (10.0%) followed by HPV-type 16 (6.7%). A systematic reviews of the literature found that the acquisition of HPV-6, 11, 16, or 18 is common among men and that the detection rate depend on the population, HPV type, and methods used [12, 20, 22]. Although it is insignificant, the results also showed that the highest infection rate was in the age group 26-32 years (8.9%). This is probably related to the high prevalence of HPV infection during the period of maximal sexual activity [22,23]. However, in USA, where HPV is the most common sexually transmitted infection, it has been estimated that 74% occurred between ages of 15 and 24 years [24]. In our community, males may delay in seeking medical help because they feel ashamed as infertility is one of community stigma diseases.

Concerning the association of HPV infection and sperm quality, high HPV infection was found among males with oligospermia and azoospermia compared to health males. These results are plausible and expected as a lot of previous studies documents the effect of HPV infection on total sperm count [10,11,25,26] . On the contrary, other studies had denied such association between HPV infection and sperm count in infertile men [27-29]. Furthermore, HPV infection, especially type 11 was found to be associated with significant reduction in sperm motility. Again several worldwide studies had obtained similar outcomes [9,26,30,31]. The more fascinating results are the significant association of HPV infection type 6, 11, and 16 with the deformity of sperm morphology. Actually, upon reviewing the literature, limited studies had point out to this issue [10,30, 31]. It has been suggested that the molecular mechanisms underlying impairment of sperm quality need further evaluations [11]. Of note, oncogenic HPV genotypes were detected on spermatozoa from symptomatic and asymptomatic subjects, though the role of the infection in male infertility was indefinitely demonstrated yet [32]. However, certain studies attributed that to the induction of anti-sperm antibodies [9]. More importantly, HPV infections are shown to be significantly associated to reduction in natural and assisted cumulative pregnancy rate and an increase in miscarriage rate are related to the presence of HPV at sperm level. Although the exact mechanism by which sperm infection is able to impair fertility remains unclear, this aspect is worthy of further investigations [13,26].

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