



Prevalence of HPV Infection and High Risk HPV Genotypes (16, 18), among Monogamous and Polygamous Women, In Zabol, Iran

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(Received 3 Jan 2011; accepted 11 Jun 2011)

Abstract

Background: This study was performed to compare the prevalence of HPV infection and high risk HPV genotypes [16, 18] between monogamous and polygamous women, in Zabol, Iran.

Methods: This cross sectional study was conducted in Zabol in 2006 – 2007. Two hundred sixty five married women attending the Gynecology Clinic for Cervical Disease Screening entered to this study. One hundred sixty two cases had monogamous, and 103 had polygamous husbands. HPV PCR samples were obtained from scrape of papsmear specimens. The biotinylated primers MY09/MY11, GP5+/GP6+, were utilized to enable amplification and detection of positive PCR products. Confirmation of HPV-16 and -18 were done by type-specific PCR primers HPV-16/F, HPV-16/R and HPV-18/F, HPV-18/R.

Results: Prevalence of HPV infection in monogamous and polygamous groups was 29% and 37.9%, respectively. The most HPV infection was found in 15-25 years group. The most prevalence of infection in monogamous group was HPV-18 and HPV- non16, 18 in 15-25 years, and HPV-16 in 26-35 years group. In polygamous group the most prevalent type was HPV-16, 18 in 15-25 years group. The most prevalent HPV-16 was seen in sever inflammation and dysplasia cytology in both groups.

Conclusion: Prevalence of HPV infection in Zabol is high, and in women with polygamous husbands group is slightly more than monogamous. Screening for this infection must be recommended in this region of Iran.

Keywords: *Human papillomavirus, Prevalence, High risk genotypes, Polygamy, Iran*

Introduction

Cervical cancer (CC) is the second most common cause of cancer death in women, and often the most common cancer in developing countries (1). Wide disparities in CC incidence and mortality treat pre-invasive disease of the cervix (2). There is consistent evidence of a causal association between certain types of human papillomavirus (HPV) and cervical intraepithelial neoplasia (CIN), a precursor of CC (3). Attributable fraction of CC due to HPV (any type) has been esti-

mated 91% in developing countries. HPV is transmitted sexually, and natural history studies have shown that the probability of young women acquiring it is high (4).

More than 50 types of HPV that infect the genital tract have been identified, and a few of them, notably HPV 16, 18, 31, 33, 45, and 58, are predominant in tissue specimens from individuals with invasive cervical carcinoma (ICC) (5). Overall, HPV is found in >90% of ICC tissue specimens (6). Approximately 70% of CCs are caused by HPV types 16 or 18. About 50% to 60% of precancer-

ous lesions CIN2 and 3 in USA is attributable to HPV16 and 18. In contrast, CIN1 is caused by a variety of HPV types, about 25% by HPV16 or 18, and 5% by HPV6 or 11 (1).

Sexual contact with an infected partner is the most common route of transmission of genital HPV. Often, the infected partner has subclinical genital HPV and is not aware that he/she is infected (7).

What are less well understood worldwide are the burden of infection in women and the natural history of HPV infection. This information is important for a better understanding of which prevention strategies, such as screening programs, public health education and vaccines are likely to be effective (4).

The limited available information on HPV in Asia confirms that peak HPV prevalence occurs in young women (8). Prevalence of HPV infection in sexually active women 20-74 yr of age, in South Korea was 10.4% (8). Prevalence of HPV infections with oncogenic subtypes in middle-aged women in China was 23.6% (2).

On the basis of epidemiological data showing that High-risk oncogenic genital HPV- 16 and 18 are the most frequently detected types in CCs worldwide (9, 10).

The distribution of HPVs varies greatly across populations and HPV surveys have been performed in different geographical regions in order to apply appropriate strategies (3).

In Zabol, people are Muslim and polygamy is culturally accepted. Thus in this study, to assess the impact of male multiple partners as a risk factor, prevalence of HPV infection, and HR-HPV [16, 18] were investigated in monogamous and polygamous women.

Materials and Methods

This cross sectional study was conducted in Zabol; north of Sistan and Baluchestan Province, Iran, in 2006-2007. Two hundred sixty five married women attending the Gynecology Clinic of Zabol Medical Sciences University for cervical disease screening entered to this study. One hundred sixty two of them had monogamous, and 103 had po-

lygamous husbands. Pap cytology was performed on cervical samples collected at clinical visits.

DNA extraction from Pap-smears

For DNA extraction, the coverslip were removed by soaking the slides in xylene over a period of 2 to 3 d. Destaining was performed immersing the slides in a series of graded alcohol solutions down to 50% ethanol. The cells were then scraped from the slides with razor blade and transferred into a 1.5-ml Eppendorf microtube containing 200 µl digestion buffer (0.2 M Tris-HCL [PH 7.5], 25 mM EDTA, 0.3 M NaCl, 2% SDS). Subsequently, 20 µg of proteinase K was add, and the cells were digested at 60° C for 2 h. the digested samples were extracted two time with phenol- chloroform and one time with chloroform, and DNA was precipitated by adding 50 µl 3M sodium acetate and 1 ml ethanol. After 1 h at -70° C, the samples were centrifuged for 10 min at 14000 rpm. The DNA pellet were dried and dissolved in 100 µl TE (10 mM Tris-HCL [pH 7.5], 0.1 mM EDTA). DNA samples were then checked by agarose gel electrophoresis to verify that degradation had not occurred during the extraction (11). In addition, the latter was confirmed by obtaining positive results for beta globin primers [PCO3, 5'-ACA CAA CTG TGT TCA CTA GC-3' and PCO4, 5'-CAA CTT CAT CCA CGT TCA CC-3'] PCR in all samples.

PCR amplification

Polymerase chain reaction (PCR) was performed by nested PCR methodology using MY09/MY11 (as outer pair) and GP5+/GP6+ (as inner) oligo-primers. Both outer and inner PCR amplification reactions were performed in a total volume of 50 µl containing 100 ng of DNA extracted papsmears, 50 mM-KCl, 10 mM Tris-HCl pH 8.3, 200 µM of each dNTP, 2 to 4 mM MgCl₂, 1 U Taq polymerase and 50 pmol of each primer. For the first-amplification step of nested PCR, 5 µl of DNA (10 to 500 ng) was used as target DNA (outer reaction); in the second step PCR, 5 µl of the first step was used as input of amplified DNA (inner reaction) (3). A sample was considered HPV

positive if one of the two amplification methods was positive, and negative if all tests were negative. The primers used in this study were MY09/MY11 and GP5+/GP6+ pairs with the following sequences [de Roba Husman et al: 1995 modified in German Cancer Research Center]:

MY09 5' CGT CCM ARR GGA WAC TGA TC 3'
MY11 5' GCM CAG GGW CAT AAY AAT GG 3'
GP5 + 5' TTT GTT ACT GTG GGT A GATA CTAC
GP6 + 5' GAA AAA TAA ACT GTA AATCA-TATTC

The mixture was denatured at 94° C for 5 min, followed by 40 cycles of amplification using a PCR processor (Eppendorf) Germany. Each cycle consisted of 94° C for 1.5 min, 40° C for 2 min, and 72° C for 1.5 min. The final elongation step was prolonged for 4 minutes to ensure complete extension of the amplified product. Samples processing prior to and after the amplification reactions were performed in strictly separated rooms to avoid contamination by PCR products. Samples containing distilled water were used as negative controls. Ten µl of each PCR mixture was finally analyzed by 1.5% agarose gel electrophoresis (3, 12).

Positive HPV DNA PCR samples were further analyzed for HPV genotyping. HPV genotyping PCR was carried out by using specific primers for amplification of the sequence containing L1 region of HPV 16 and 18; with the following sequences: HPV 16 forward, 5'-TCA AAA GCC ACT GTG TCC TG-3' and reverse 16, 5'-CGT GTT CTT GAT GAT CTG CA-3' and HPV 18, forward, 5'-GAC ACA TTG GAA AAA CTA AC-3' and reverse 18, 5'-TAG TGC CCA GCT ATG TTG TG-3'. The amplified products correspond to 119bp for HPV 16 and 139bp for HPV 18. PCR reaction with type specific primers was performed in a total volume of 25 µl containing 2.5 µl of 10X PCR buffer, 2 mM MgCl₂, 200 mM of each dNTPs, 1U of Taq DNA polymerase, 20 pmol of each primer (a mixture of HPV-16 and HPV-18 specific primers). Hela cell DNA extracts and distilled water were used as positive and negative controls respectively. After holding at 94° C for 3 min the mixture was subjected to

30 cycles of PCR amplification (denaturation at 94° C for 1 min, annealing at 58° C for 1 min, and extension at 72° C for 1min). The last cycle was followed by an additional 5 min of extension at 72° C. The amplified fragments were resolved by electrophoresis on the 2% agarose gel in 80 volts for 90 min, stained with ethidium bromide, and visualized on a UV (12).

Statistical Analysis

Data were analyzed using descriptive statistics and analytical (chi squared χ^2 test) to examine the prevalence of HPV infection and high-risk genotypes (HPV-16, 18) and comparison of it between groups. Differences were considered statistically significant when *P* values were less than 0.05.

Results

From 265 women entered to this study, 162 women had monogamous husbands (mean age 28.58±8.21 yr) and 103 women had polygamous husbands (mean age 27.32±6.52). The mean age of first sexual intercourse was found to be comparable in monogamous and polygamous groups (18.2±5.3 versus 17.6±3.8).

Prevalence of HPV infection in monogamous and polygamous group was 29% and 37.9%. Prevalence of HPV+ in polygamous group was higher than monogamous, although this difference was not statistically significant (*P*> 0.05) (Table 1). In both groups the most HPV infection was found in 15-25 yr old (respectively 51.3% and 38.3% in polygamous and monogamous groups).

In monogamous group, the most prevalent infections of HPV-18 and other types of HPV were seen in 15-25 yr age subgroup (respectively 38.5% and 40% of all positive HPV-18 and other types of HPV). HPV-16 was most prevalent in 26-35 yr age subgroup (50% of all positive HPV-16).

In polygamous group, the most prevalent infections of HPV-16 and HPV-18 were seen in 15-25 yr age subgroup (respectively 77.8% and 63.6% of all positive HPV-16 and HPV-18 were in this subgroup) (Table 2). In this table, "n stands for number of HPV type and (%) its percent in each age subgroup".

The most HPV infection in monogamous group was seen in severe inflammation with or without dysplasia (51.4%), and in polygamous group was seen in dysplasia with moderate or severe inflammation (58.3%). The most prevalent HPV-16 was seen in severe inflammation and dysplasia in both groups (Table 3).

Majority of HPV+ monogamous wives had 1-5 pregnancies (51.1%), whilst majority of polyga-

mous wives had no pregnancies (89.7%). In HPV-16 + monogamous wives, 64.3% had 1-5 pregnancies, whilst in HPV-16+ polygamous wives 88.9% had no pregnancies (Table 4). It must be mentioned that in our study infertility was the main problem of 61.5% of polygamous and 23.4% of monogamous wives.

Table 1: Distribution of human papillomavirus infection in women with monogamous and polygamous husbands, relative to age in Zabol

Groups	HPV	Age subgroups (yr)				Total n (%)
		15-25 n (%)	26-35 n (%)	36-45 n (%)	>45 n (%)	
Monogamous	+	18(28.1)	17(29.3)	8(25.8)	4(44.4)	47(29)
	-	46(71.9)	41(70.7)	23(74.2)	5(55.6)	115(71)
Polygamous	+	20(44.4)	15(31.3)	4(50)	0(0)	39(37.9)
	-	24(55.6)	33(68.6)	4(50)	2(100)	64(62.1)

Table 2: Distribution of human papillomavirus genotypes in women with monogamous and polygamous husbands, relative to age in Zabol

Groups	Age subgroups (yr)	Human Papilloma Virus genotype				Total n (%)
		HPV+			HPV- n (%)	
		HPV-16 n (%)	HPV-18 n (%)	Other types n (%)		
Monogamous	15-25	5(35.7)	5(38.5)	8(40)	46(40)	64(39.5)
	26-35	7(50)	4(30.8)	6(30)	41(35.7)	58(35.8)
	36-45	1(7.1)	4(30.8)	3(15)	23(20)	31(19.1)
	>45	1(7.1)	0(0)	3(15)	5(4.3)	9(5.6)
Polygamous	15-25	7(77.8)	7(63.6)	6(31.6)	25(39.1)	45(43.7)
	26-35	2(22.2)	4(36.4)	9(47.4)	33(51.6)	48(46.6)
	36-45	0(0)	0(0)	4(21.4)	4(6.3)	8(7.8)
	>45	0(0)	0(0)	0(0)	2(3.1)	2(1.9)

Table 3: Distribution of different HPV genotype infections related to cytological results in women with monogamous and polygamous husbands, in Zabol

Groups	Cytological Results	Human Papilloma Virus Genotype				Total n (%)
		HPV+			HPV- n (%)	
		HPV-16 n (%)	HPV-18 n (%)	Other types n (%)		
Monogamous	Moderate inflammation	3(21.4)	4(30.8)	9(45)	46(40)	62(38.3)
	Sever inflammation	1(7.1)	3(23.1)	3(15)	7(6.1)	14(8.6)
	Moderate inflammation and dysplasia	2(14.3)	2(15.4)	2(10)	45(39.1)	51(31.5)
	Sever inflammation and dysplasia	8(57.1)	4(30.8)	6(30)	18(14.8)	35(21.6)
Polygamous	Moderate inflammation	2(22.2)	2(18.2)	6(31.6)	24(37.5)	34(33)
	Sever inflammation	0(0)	0(0)	2(10.5)	2(3.1)	4(3.9)
	Moderate inflammation and dysplasia	2(22.2)	5(45.5)	6(31.6)	28(43.8)	41(39.8)
	Sever inflammation and dysplasia	5(55.6)	4(36.4)	5(26.3)	10(15.6)	24(23.3)

Table 4: Distribution of different HPV genotypes related to number of pregnancies in women with monogamous and polygamous husbands, in Zabol

Groups	Number of pregnancies	Papilloma Virus genotype				Total n (%)
		HPV+			HPV- n (%)	
		HPV-16 n (%)	HPV-18 n (%)	Other types n (%)		
Monogamous	Non	3(21.4)	4(30.8)	7(35)	49(42.6)	63(38.9)
	1-5	9(64.3)	6(46.2)	9(45)	47(40.9)	71(43.8)
	6-10	2(14.3)	3(23.1)	4(20)	19(16.5)	28(17.3)
Polygamous	Non	8(88.9)	11(100)	16(84.2)	54(84.4)	89(86.4)
	1-5	1(11.1)	0(0)	2(10.5)	7(10.9)	10(9.7)
	6-10	0(0)	0(0)	1(5.3)	3(4.7)	4(3.9)

Discussion

We evaluated, for the first time, prevalence of HPV infection and HR-HPV (16, 18), in women with monogamous and polygamous husbands in Zabol; north of Sistan and Baluchestan province, Iran.

Little is known about the HPV prevalence in Asia (5). In addition to expanding our knowledge of the worldwide distribution of HPV; this study provides valuable information on the feasibility of surveys and clinical trials that target HPV infection, in Iranian women.

In our study, prevalence of HPV infection in monogamous and polygamous wives was 29% and 37.9%. HPV prevalence in USA among 14-59 yr old women was 26.8% (13). In Taiwanese 16-78 yr old women 19.3% (14), in Colombian 20-55 yr old 11.4% (15), in Costa Rican 18-94 yr old 7.6% (16), in Mexican women aged 35-64 yr 4-12% (17), and in West African immigrants in south Italy was 42.2% (3).

In Asian surveys, prevalence rates of HPV infections were: 10% in South Korean women aged 20-74 yr (8), 10.9% in South Vietnamese women aged 25- 65 yr (18), 6.3% in Thailand in women aged 15 yr (8), and 23.6% positivity for oncogenic subtypes in china in middle-aged women (2).

Prevalence of HPV+ in Holland was 25.4% (19), in Nigeria 26.3% (20) and in Canada 24.0% among 20-24 yr of age that was progressively lower in older ages, reaching 3.4% in 45-49 yr old (4).

Prevalence of HPV infection in Zabol is higher than abovementioned countries. HPV prevalence in polygamous was higher than monogamous group, and was comparable to prevalence of 40% in Mozambique (21), 31% in Zimbabwe (22), 34% in Tanzania (9) and 44% in Kenya (23).

HPV infection in Tunisia was less frequent than in other African countries (14% in married women) (24). In Bahrain there was 11% positivity with HR-HPV subtypes (25).

Based on several studies the most significant risk factor for HPV infection is multiple lifetime sex partners (1-4, 7, 26). In Algeria, husband's extra-marital sexual relationships was directly associated with HPV infection in their wives (27).

Having numerous sexual partners increases an individual's likelihood of HPV exposure (26, 28).

It has been shown that increased number of sexual partners or common use of prostitutes by husbands affects the probability of HPV transmission to female partner. This effect has been particularly described in populations in which monogamy was common (28).

In present study, to assess the impact of multiple partners as a risk factor, polygamy, which is culturally accepted in this Muslim population, was used as a measure of the effect of male sexual behavior.

A study in Mali showed that 58% of controls and 77% of CC cases had polygamous husbands. Polygamy was common among women with CC and cancer cases were more likely to share a husband with more than two wives. Polygamy in-

creased by twofold the risk of CC, but the estimate was at limit of statistical significance. This risk increased with the number of wives within the family (28). They had almost no data directly provided by husbands, and thus could not control for the sexual behavior of the husband. Finally, they concluded that the adverse effect of polygamy in their population might well reflect higher promiscuity of the husbands.

In Mali similar to Zabol, age of first sexual intercourse of women was exactly age of marriage and reflects the facts that in this sociocultural setting, onset of sexual intercourse takes place within the context of marriage, which tends to occur at an early age and a woman having multiple partners is generally unusual. Increase of likelihood of HPV infection at the time of first intercourse due to the husband's previous sexual experiment with other wives might be a key determinant of the HPV infection among young women with polygamous husband (28).

In agreement with our study, in Bahrain, polygamy was not identified as a risk factor (25).

In our study the most HPV infection was found in age of 15-25 yr in both groups. Available information on HPV in Asia confirms that peak of prevalence occurs in young women (2, 8, 25). Prevalence of HPV in Canada was highest in young women and progressively lowered in older women (4). Development of an immune response has often been suggested as the explanation for decreasing prevalence of HPV infection with increasing age (24, 29).

Our study showed that in monogamous group the most prevalence infection was HPV-18 in 15-25 yr, and HPV-16 in 26-35 yr age. In polygamous group the most prevalent types were HPV-16 and HPV-18 in 15-25 yr. Based on epidemiological data, HPV-16 and 18 are the most frequently detected types in CCs worldwide (9). Many studies in different provinces in Iran have evaluated the prevalence of HPV in women with cervical cancer, but the findings were not similar. In a study in Iran (Yazd) 75% of CCs specimens were HPV+ and the most prevalent genotype were respectively HPV-16 (70%) and HPV-18

(16.8%) (30). Another study in north of Iran showed that 74.4% of cancer case, 66.6% of Dys/Metaplasia specimen and 8.8% of normal specimens were HPV+ and 51.1% of HPV+ were 16/18 genotypes(31). Whilst, Farjadian et al., in a study in Shiraz showed that 87.1% of the CC samples were HPV+ and HPV-16/18 were not the frequent high-risk HPV types (12). Esmaili et al in a study in northwest of Iran showed that the most prevalent oncogenic HPV was type 16 followed by types 31, 18 and 33. Multiple HPV infections were present in 15.3% of the samples (32). In Bushehr, Zandi et al. also showed that HPV-16 is the most prevalent type among high risk HPV (33). The findings of Al-lame et al. in Isfahan also indicated a positive rate of 90.8% of HPV infections among women with abnormal cytology of cervix. The high-risk subtypes of HPV, i.e., 16 and 18, were the dominant types (34).

The results of our study were in line with earlier studies in Isfahan (34), Shiraz (12), Yazd (30), Tehran (35-37) Mazandaran (31), but different from those in Bandarabas (38) Tehran (39) and Isfahan (40).

In Tanzania the most common genotypes were HPV-16, 58, M7, 33, and 18 (9). In West German area, the most frequent types were HPV-16, 31 and 18 (10). The most prevalent genotype among women in Mozambique was HPV-35 (21), in Kenya HPV-52(23), in Nigeria HPV-16 and 35 (20), in Taiwan HPV-16, 52, and 58 (14). In Tunisian women, the most prevalent type in prostitutes was HPV-16 and in married women was HPV-6, which is associated with a low risk for CC (24).

In West Africa, the most frequently detected HPV types were 16 and 58 (41). But in West African immigrants in south Italy the most common viral types were other than 16 and 18 (3).

In Algeria HPV- 16 was the most common type, followed by HPV- 18 and 45. HPV- 16 or 18 was found in nearly 70% of cases and in more than half of HPV+ cytologically normal controls (27).

In HSIL cases in Zimbabwe, HPV-16, 58, 18, and 52 were the most common genotypes, but

these women had a high prevalence of HIV (78%) and multiple HPV infections (68%) (22).

After HPV-16, the second most prevalent subtype is HPV-18 in Western countries and HPV-58 in Asia (5).

A study on prevalence of HPV genotypes in routine papsmears of Korean women showed that HPV DNA was found in 44.8% of the total cases and 58.7% of the atypical lesions. Three major HPV genotypes identified were HPV-16, 52, and 58 (6). They found that frequency of HPV-52 and 58 is increasing in cervical malignancies.

With due attention to high prevalence of viral types other than HPV16, 18 in monogamous (43%) and polygamous wives (48%) in Zabol, further work is needed to identify unknown genotypes other than 16, 18 in these women.

Our studies showed that the most prevalent HPV-16 was seen in sever inflammation and dysplasia cytology in both groups. Hajjaje et al., have demonstrated normal cervical cytology in HPV+ women (25). Other studies showed that infection with multiple HPV types has been observed more frequently among women with cytological abnormalities (12, 13, 19).

Speich et al. showed that the results of the HPV tests were in good agreement with cytological diagnosis. Women with HSIL all showed infection with high-risk HPV types, which were mostly HPV 16, 18, 31, 33 or 66 (10).

There is some epidemiological evidence suggest that genital tract disease such as cervical inflammation might be linked to cervical cancer or high-grade lesions (2).

The reason for low prevalence of CC HPV+ women in our study might be that CIN does not develop in the majority of young women who acquire genital HPV. It was found a 36 mo cumulative incidence of 20% for CIN- 2 compared with 6.7% for CIN- 3 among women with HPV-16 or 18. A minority of mild cases of CIN either persists without further progression or progresses to cancer over several years (7).

Because of high prevalence of HPV infection in Zabol, further works are needed to identify unknown genotypes other than 16, 18 in women,

and prevalence of HPV genotypes in men in Zabol. This information will increase our understanding of the natural history of HPV infection and assist in the development of effective screening programs.

Ethical Considerations

Ethical issues including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc. have been completely observed by the authors.

Acknowledgments

We are grateful to the research council of Zabol University of Medical Sciences, who supported financially this work. We also acknowledge Dr. Yaghmaei M, and Dr. Sharifi- mood B, and Dr. Heidari F. for their helps. The authors declare that there is no conflict of interests.

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