

RESEARCH ARTICLE

Prevalence and Genotype Distribution of Cervical High-Risk Human Papilloma Virus in Cervical Specimens of Woman with Normal and Abnormal Cytology in Anbar province, Iraq

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ABSTRACT

Background: Cervical High-Risk Human Papillomaviruses (HR-HPVs) is used as a marker for early cervical cancer (CC) detection as these viruses are the leading cause of CC. Therefore, the detection of these viruses is very necessary for the use of prophylactic methods against this cancer and other diseases associated with these viruses.

Objective: To know the prevalence and genotype of cervical HR-HPVs in normal and abnormal cervical lesions of women attending Al-Ramadi Maternity and Children's Teaching hospital, Iraq.

Material and Methods: Descriptive cross-sectional study was done on 339 women with cervical lesions in AL-Ramadi Maternity and Children's Teaching in cooperation with Anbar Medical College, Ramadi, Iraq, during a period from August 2019 to February 2020 to detect the prevalence of different HPV types in women with or without cervical cytological abnormalities using "clinical Pap smear tests" in addition to "Xpert-HPV procedure".

Results: Cervical HR-HPVs were confirmed in 12.9% of all patients, of them, 5.0% were cervical HR-HPV-18-45 followed by cervical HR-HPV-16 (presented in 4.4% of the patient), followed by other HR-HPV 11 (3.5%). Multiple cervical HR-HPV types had been detected in 6.1% of the positive confirmed patients. There was a statistically significant difference with regard to the prevalence of cervical HR-HPV types within different types of histological smears (P Value 0.000) and that some HPV types spread in the normal cytological smears of the infected woman.

Conclusion: The prevalence of cervical "HR-HPV" in 339 patients included in the study was 12.9%. Cervical HR-HPVs 16, 18 - 45 & other HR-HPV were the HR-HPVs of current study. This requires the inclusion of vaccines against these viruses in the prophylaxis program in Iraq.

Keyword: Human papillomavirus, cervical smear, cervical cancer, prevalence, Anbar province, Iraq

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INTRODUCTION

The prevalence of cervical HPV worldwide is 10-22%, Iraqi patients are not free from this virus but have registered low prevalence.¹ Certain HPVs are 'Low risk' (LR-HPV) as it only responsible for benign dermal warts, but high risk HPV (HR-HPV) such as can HPVs 16 and 18 and other subset of 14 of these types responsible for malignant form of cancers, particularly cervical cancer (CC).²

CC is the 2nd most predominant reason of death due to cancer among women with 52% global mortality rate and the 3rd most common cancer worldwide,³ it is one of the top ten cancer cases in America,^{4,5} and fifth in China, with 30,000 deaths in 2015.⁶ In the Arab Gulf countries, CC is one of the top 11 cancer cases with 3.0 per 100,000 women incidence rate, whereas in Iraq, the incidence rate was 7.4 per 100,000 women.⁷

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Individuals with clinically invisible cervical HPV infection can transmit the virus through sexual contact and this has helped to increase the rate of infection.^{4,5}

The Xpert HPV Assay is a qualitative in vitro test for E6/E7 region detection of HR-HPV DNA genome in patient specimens. The test carries out multiplexed amplification of target DNA by real-time Polymerase Chain Reaction (PCR) a single analysis⁸ to identifies HPV16 and HPV 18/45 types in two distinct detection channels, and reports 11 other high risk types in squamous intraepithelial lesions of women cervical lesion (31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68) in a pooled result.⁹

Cervical HR-HPV prevalence woman give us essential data on distribution of cervical HR-HPV infection in Iraqi as these data help health institutions in deciding to conduct periodic vaccines against this virus in Iraq, which is reflected in reducing cases of cervical cancer, as this virus is the most important cause.

PATIENTS AND METHODS

In this cross sectional study, 339 women with or without squamous intraepithelial cervical lesions attending the Gynecology Clinic of AL-Ramadi Maternity and Children's Teaching hospital in cooperation with Anbar Medical College, Ramadi, Iraq, during the period from August 2019 to February 2020 to show the prevalence of cervical HR- HPV types, in addition to cytological and molecular results through using of "clinical Pap smear tests" & "Xpert HPV" Assay respectively. Woman had been admitted to the Maternity hospital after abnormal bleeding associated with painful uterine discharge during intercourse cervical smears were unusual.

The "inclusion criteria": "Genital warts", uterine & cervix microscopically suspicious" linked with HPV, "Pap smear result suspicious for "cervical HR-HPV", "HPV + partner had been included in current study.

"Exclusion criteria": pregnant women, Hysterectomy had been exclude during the study period.

A "questionnaire data form" had been designed regarding "socio-demographic data, patient's" as medical history & other threat factors such as Smoking habit, age, "sexual exposure" in years, "age at 1st sexual interaction" and "marital-status", had been managed by the physician for all "participants patients".

Pap smears had been read at the "department of Cytology" in AL-Ramadi Maternity & Children's Teaching hospital, Iraq, & these smears had been ordered as describe in Table 5.

Conformation had been done by two consultant in pathology using of 2002- Bethesda-classification as a reference.

Finding & typing cervical HR- HPV DNA was carried out using Xpert HPV Assay (Cepheid company, USA) according to the manufacturer's instructions. Family consent was obtained from all patients. Research was approved by the Scientific Ethics Committee of the Anbar medical college, Anbar University.

Statistical Analysis

Statistical analysis had been done through using SPSS "by "discreptional-analysis" & "Cross-tabular" recognize socio-

demographic data linked with "cervical HR-HPV" prevalence. The 95% CIs had been adopted to regulate the "statistical significance" that had been assumed at the "p < 0.05" levels.

RESULTS

Socio-demographic Data

The study group was distributed out as follows: Ramadi (n = 228; 67.3%), Fallujah (n = 75; 22.1%), Heet (n = 24; 7.1%), and Haditha (n = 3.5; 3.5%), women. 206, (60.8%) of them were from Urban and 133 (39.2%) from Rural in Anbar province (Table 1).

Over 91.2% of cervical HR-HPV⁺ women were married, 7.1% Separated, 0.9% Widowed and 0.9% Polygnous marriage (Table 1). Neither Smokers nor "sexual-partners" number appear to be a significantly correlated with cervical HR-HPV infection as 86.4% of those women have one sexual partner and 92.9% of women were non-smokers. Neither condom use nor Wealth Index appear to be a significantly correlated with cervical HR-HPV infection as 322 (95.0%) of them did not use a condom. Of woman, 172 (50.7%) and 119 (35.1%) had Middle and Low Wealth Index respectively. Of 399 woman, 238 (70.2%) had in secondary school and 309 (91.2%) were housewives. Infection with all cervical HR-HPV occurred though sexual contact. There were a statistically significant difference with regard to the prevalence of different types of HPV within the categories of women with varied educational attainment (P. Value 0.000) as presented in Table 1.

Cervical HR- HPV-positive women mean age was 34.26 years (ranging from 23 to 45 years). There were no statistically significant difference in mean ages of cervical HR- HPV-positive women infected with different HR-HPV types (P. Value, 0.853) as shown in Table 2.

Out of the HPV-positive women, 16 women (37.8%) had been sexually active for 11–18 years, 11 women (26.5%) had been sexually active for <= 10 years 17 women (35.7%) were "sexually-active" for up to 19 years. Majority cervical HR-HPV⁺ women the were up to 23 years as shown in Table 3.

Among the 339 patients, 12.9% (n = 32) were positive for HPV DNA that distributed between in patients aged <= 34 (53.1%) and 35+ (46.9%) years.

Cervical HR-HPV-18 - 45 was predominant types noticed (in 17 women; 5.0%), of them 9 (52.9%) in the age group <= 34 and 8 (47.1%) in the age group 35+; followed by HPV- 16 in 15 (4.4%), of them 8 (53.3%) in the age group <= 34 and 7 (46.7%) in the age group 35+ and other HR-HPV in 12 woman (3.5%), of them 4 (33.3%) in the age group <= 34 and 8 (66.7%) in the age group 35+. Multiple HPV types infections were detected in 11 women (6.1%). whereas 86.9% of women in the study were free from infection with this virus as shown in Table 4.

Cytology and Xpert HPV DNA assay

Of 399 woman, 307 (90.6%) with normal cytological smears, 32 (9.4%) with abnormal cytological smears. Of 32 (9.4%) women with abnormal cytological smears, 9 (2.7%) had CC (all of them were positive for HPV DNA), 11 (3.2%) LGSIL (of them, 9 were cervical HR-HPV DNA⁺), 4 (1.2%) HGSIL

Table 1: Socio-demographic data of women with normal and abnormal cervical cytology

Character	Number (%)	Character	Number (%)
<i>City</i>		<i>Marital status</i>	
Ramadi	228 (67.3)	Married	309 (91.2)
Fallujah	75 (22.1)	Separated	24 (7.1)
Heet	24 (7.1)	Widowed	3 (0.9)
Haditha	12 (3.5)	Polygynous marriage	3 (0.9)
<i>Residency</i>		<i>Smoking</i>	24 (7.1)
Urban	206 (60.8)	Yes	
Rural	133 (39.2)	No	315 (92.9)
<i>Educational attainment</i>		<i>Occupation</i>	
Secondary school	238 (70.2)	House wife	255 (75.2)
Primary school	58 (17.1)	Employee	64 (18.9)
University student	42 (12.4)	Teacher	18 (5.3)
Holds university	1 (0.3)	Doctors	2 (0.6)
<i>Age Group</i>		<i>Condom marital</i>	
<= 34	180 (53.1%)	Yes	17 (5.0)
35+	159 (46.9%)	No	322 (95.0)
<i>Wealth Index</i>		<i>Sexual partner</i>	
Middle	172 (50.7)	One	293 (86.4)
Low	119 (35.1)	Two	41 (12.1)
High	48 (14.2)	Three and more	5 (1.5)
<i>Condom marital</i>		<i>Year of sexual exposure</i>	
Yes	17 (5.0)	<= 10	90 (26.5)
No	322 (95.0)	11 - 18	128 (37.8)
		19+	121 (35.7)

Table 2: Cervical HR- HPV-positive women mean age infected with different HPV types

	N	Mean	Std. Deviation	P Value
16	15	33.87	7.405	
18 - 45	17	34.82	7.100	
Other High risk	12	35.75	5.723	0.853
Negative	295	34.19	6.746	
Total	339	34.26	6.739	

Table 3: Frequency of HPV types among woman cervix depending on years of sexual exposure

Type HPV	Years of sexual exposure group			Frequency (%)
	<= 10	11 - 18	19+	
16	5	5	5	15 (4.4)
	33.3%	33.3%	33.3%	100.0%
18 - 45	4	6	7	17 (5.0)
	23.5%	35.3%	41.2%	100.0%
Other High risk	2	5	5	12 (3.5)
	16.7%	41.7%	41.7%	100.0%
Negative	79	112	104	295 (87.0)
	26.8%	38.0%	35.3%	100.0%
Total	90	128	121	339 (100.0)
	26.5%	37.8%	35.7%	100.0%

(all of them, were cervical HR-HPV DNA⁺), 3 (0.9%) had Intraepithelial Neoplasia (all of them were cervical HR-HPV

Table 4: Frequency of HPV types in women cervix according to age group

Type HPV	Age Group		Frequency (%)
	<= 34	35+	
16	8	7	15 (4.4)
	53.3%	46.7%	
18 - 45	9	8	17 (5.0)
	52.9%	47.1%	
Other High risk	4	8	12 (3.5)
	33.3%	66.7%	
Negative	159	136	295 (87.0)
	53.9%	46.1%	
Total	180	159	339 (100.0%)
	53.1%	46.9%	

DNA⁺), 3 (0.9%) appear as ASCUS (one of them only was HPV DNA positive while others two were negative for HPV DNA), 2 (0.6%) had SCC (one of them were HPV 18–45 DNA positive). There were a statistically significant difference in HR-HPV prevalence within different types of histological smears (P. Value 0.000) and that some HR -HPVs detected in the woman normal cytological smears as cervical HR- HPV prevalence were 12, 9, and 4.7% in with & without cervical abnormalities women, respectively shown in Table 5

There is a statistically significant difference in the HR-HPVs prevalence with the number of marriage as 39 of

Table 5: HPV type's distribution among cervical smears of normal and abnormal cytology

Type HPV	Type Cytological smear							Frequency (%)
	Cervical Cancer	Intraepithelial Neoplasia	Atypical squamous cell of undetermined significance (ASCUS)	Low- grade squamous intraepithelial lesion (LGSIL)	High- grade squamous intraepithelial lesion(HGSIL)	Squamous cell carcinoma	Normal cytology	
16	5 (33.3%)	2 (13.3%)	0 (0.0%)	2 (13.3%)	2 (13.3%)	0 (0.0%)	4 (26.7%)	15 (4.4)
18-45	2 (11.8%)	0 (0.0%)	1 (5.9%)	5 (29.4%)	1 (5.9%)	1 (5.9%)	7 (41.2%)	17 (5.0)
Other High risk	2 (16.7%)	1 (8.3%)	0 (0.0%)	2 (16.7%)	1 (8.3%)	1 (8.3%)	5 (41.7%)	12 (3.5)
Negative	0 (0.0%)	0 (0.0%)	2 (0.7%)	2 (0.7%)	0 (0.0%)	0 (0.0%)	291 (98.6%)	295 (87.0)
Total	9 (2.7%)	3 (0.9%)	3 (0.9%)	11 (3.2%)	4 (1.2%)	2 (0.6%)	307 (90.6%)	339 (100.0%)

Table 6: Association of HPV type with number sexual partners among woman with cervical lesions

Sexual partners	Type HPV				Total	P.value
	16	18 - 45	Other High risk	Negative		
One	14 4.8%	14 4.8%	11 3.8%	254 86.7%	293 100.0%	0.750
Two	1 2.4%	2 4.9%	1 2.4%	37 90.2%	41 100.0%	
Three and more	0 0.0%	1 20.0%	0 0.0%	4 80.0%	5 100.0%	
Total	15 4.4%	17 5.0%	12 3.5%	295 87.0%	339 100.0%	

single-time married women, 4 of two times married women and only one of three and more times of married women had different HR-HPVs (P. Value 0.750) as presented in Table 6.

DISCUSSION

Xpert HPV assay was done for the first time to determine cervical HR-HPV prevalence in women with and without cervical abnormalities of women residing in Al-Anbar province Iraq.

Cervical HR-HPV typing results demonstrated that HPV 18-45 and HPV-16 to be the most prevalent types during current study (Table 4), these results were consistent with study performed in western region of Asia, wherever Iraq country belongs to, that showed the prevalence rates of HPV-18, HPV 16 were 4.4 % and 5.0 % respectively and also HPV19-45 were detected during this study in addition to 2.3% of cervical HR-HPV-16/18 had been detected in over-all women population.⁷ In contrast to other study that showed 72.4% of HPVs 16 or 18 found in invasive CC.¹⁰ Current study was in contrast to the previous studies that showed cervical HR-HPVs 33, 35, 56, 39 & 52 were appeared to be more predominant than cervical HR-HPVs 16 & 18 in Baghdad, Iraq & sometime with more than one type of HPV.¹¹

We found that prevalence of cervical HR-HPVs in women with & without cervical abnormalities were 12.9 % & 4.7% respectively through using "Xpert-HPV" Assay "As shown in Table 5". This result is in the midst of widespread prevalence worldwide (9.8% -86%) Which includes "Iraq & neighboring countries".^{7,12,13}

Cervical HR-HPV prevalence during in women current study were relatively lower comparing with cervical HR-HPV prevalence that had been registered in 360 (78%) & 60 (53%) of Ethiopian & Egyptian women respectively^{7,13} and its slightly lower than that in a large previous 8-year study registered by Pity *et al.* (2012)⁷ of 10,338 north of Iraq women anywhere it was 18% & it is higher than what has been registered on 300 Canadian women by Hamlin-Douglas (2008) as the prevalence was (20.4%).¹⁴ The explanation for this case could be up to surveyed population behavioral appearance & practical method / procedure for virus identification.^{10,13,15}

In fact, all women participating in present study were women who are legally married and do not have any illegal sex due to conservative society traditions in addition to the teachings of the Islamic religion that do not hear the practice of illegal sex, This may be the reason for the low prevalence of sexually transmitted cervical HPV, On the other hand, Vaccination against cervical HR-HPV among Canadian women that made the infection rate lower than Iraqi non-vaccinated women.¹⁴

Cervical HR-HPVs had been identified in 4.7% of women without cervical abnormalities smears, Which is close to the "lower-third" of the world's prevalence (0%-60%).^{3,7,10,11,12}

Prevalence rate 3.2, 2.7 and 1.2 % of HPV DNA detection in LGSIL, CC and HGSIL respectively during current study (Table 5) was in contrast to results reported previously,^{16,17,18} Although Al-Awadhi *et al.*, (2013)¹⁵ have also reported an increase in cervical HR-HPV with lesion severity among Kuwaiti women.

The detection of 4.7 % HR-HPV amongst women without cervical abnormalities in these two categories during current

study indicates the importance of detection of this virus in woman without cervical cancer as a risk factor for cervical cancer had been similar to other previous studies, which registered cervical HR-HPV infection in normal cervical cytological lesion,¹³ But it was in consistent with other similar studies that showed t (13.9–64%) of cervical HR-HPV were identified in women with normal cytology.^{19,20}

The detection negative HR-HPV amongst women with or without cervical abnormalities for several reasons, the most important of which is due to other strains appearance (not involved in the assay), or the HR-HPVs not responsible for disease, or because the patients had been previously receiving antiviral drugs or the virus within LR-HPV.

Cervical HR-HPVs infection in young women commonly tends to be decline automatically, however, the virus more commonly appear to be persistent in older women,^{21,22} it was not clear in the current study of married women, as the risk of infection with HPV was equal in all age groups included in the study as shown in Table 2, Table 4, in contrast with other studies that showed a decrease in cervical HR-HPV infection with age group.^{23,24} This discrepancy may be caused by population sexual exposure happen after marriage that usually occur with age advancing such as women involved in current research (mean age = 35 years), whereas it was 24 years during previous study¹⁹ or mean age for women with cervical HR-HPV in first intercourse was not available.

CONCLUSION

High prevalence of cervical HR-HPVs 16, 18-45 other cervical HR-HPV in four types of cervical cytological abnormalities in addition to women without cervical abnormalities give strong proof that Anbari women are at risk of cervical cancer developing & so the vaccination prophylaxis against these viruses become very important. Developing countries should include Xpert HPV Assay in the cytology protocol in general and private hospitals for CC prophylaxes.

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