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Human papillomavirus genotypes in cervicitis and atypical squamous cell of undetermined significance lesions among women in Erbil, Iraq

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Abstract

Background and objective: Human papillomavirus infection is known to be the primary reason for cervical carcinoma. Steady infection with high risk (HR)-HPV strains is behind most abnormal cervical lesions, this studyaims to investigate the prevalence and genotype distribution of HPV in cervical samples from women between 20 to 50 years old with different cervical lesions, including cervicitis and atypical squamous cells of undetermined significance (ASC-US) cases.

Methods: Cervical smears of 113 patients diagnosed with atypical squamous cell of undetermined significance and cervicitis were included in the study and examined for HPV detection used reverse transcription polymerase chain reaction and hybridization method, followed by HPV genotyping assay specimens.

Results: Out of the total 113 cases, 24.74% were positive for HPV infection, and 75.22% were negative for HPV infection. Most of the patients diagnosed with cervicitis were within 35-39 age group, accounting for 26.3% of the total cases. In contrast, most patients with ASCUS were in the 30-34 age group, representing 29.7%. The majority of patients exhibit a single serotype (60.7%), while multiple serotypes were found in 39.3%. HR-HPV16 and multiple genotype HPV (31,35,39,51,52,56,58,59,66, and 68) were the most frequently identified genotypes.

Conclusion: The detection of both low- and HR- HPV genotypes were reported and not uncommon among patients with cervicitis and a typical squamous cell of undetermined significance. High risk (HR)-HPV16 and multiple genotypes were the most frequently diagnosed by PCR test. Consequently, it is highly advisable to create a program for cervical cancer screening and prophylactic HPV vaccination for early diagnosis and prevention of cervical abnormalities and neoplasia in Iraq.

Keywords: Human papillomavirus, Genotyping, Papsmear, Cervical cancer, Erbil Kurdistan Region-Iraq.

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Cervical cancer ranks as the fourth most prevalent cancer among women globally. It is a disease marked by inequity, as it disproportionately impacts women in low- and middle-income countries, where approximately 94% of cervical cancer-related deaths occur (1). These differences in the burden of cancer are mostly, but not entirely, due to differences in exposure to risk factors for cancer, and are not simply the result of differences in the availability of cancer treatment. Efforts to eliminate cervical cancer in Iraq and the Kurdistan Region face significant challenges due to widespread poverty and persistent gender inequality, which intensify the health burden in these areas (2). Human papillomavirus (HPV) infection is known to be the essential but not sufficient cause of cervical cancer. Nearly all cervical cancer cases are associated with sustained infection with high-risk types of HPV. However, other factors-including an individual's immune response, as well as genetics and the environment- are usually needed to accompany HPV infection for cancer to occur (3, 4). The International Agency for Research on Cancer (IARC) has found specific types of HPV to be Group1 carcinogens, which means that there is evidence of their carcinogenicity to humans. These are types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 (3). The single most important cause of cervical cancer is persistent chronic infection with a high-risk human papillomavirus (HPV) type.

A polymicrobial infection between high-risk HPV and high-risk HPV type 16 and type 18 were responsible in 71% cervical cancer cases in the world (5). The Pap smear test, a most commonly used mass screening test for pre cancer lesions in cervical cells, and Bethesda system (TBS) is used as a standard reporting format. The Bethesda system reports epithelial cell abnormality, which could be squamous cell abnormality or glandular cell abnormality (6). The technique most employed in the screening is a cervical cytological smear due to its simplicity, effectiveness, rapidity and inexpensiveness (7). A comparative study at a tertiary maternity hospital in Erbil similarly found Pap smear screening to have high sensitivity for detecting cervical intraepithelial neoplasia and early cervical cancer (25). Cervicitis an inflammation of the cervix that is most commonly caused by irritation (chemical or procedural) or infection. Infectious causes are most commonly sexually transmitted infection:the most frequent pathogens are chlamydia trachomatis and Neisseria gonorrhoeae the less frequently is human papillomavirus (HPV) (8). ASCUS (Atypical squamous cells of undetermined significance)is a based on equivocal morphologic findings and can include the aforementioned but adds the category

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of “Atypical Squamous Cells” (ASCs), This is used when there are abnormalities of squamous epithelium that are more marked than those of a reactive process but does not meet the criteria squamous intraepithelial lesion(SIL). 10-20% of women with ASC-US results may have high-grade lesions on follow up (7, 9). Human papilloma viruses (HPV) are a large family of over a hundred viruses that are linked to a diverse group of mucocutaneous diseases, from benign warts present at multiple sites and among many subpopulations to possibly articles, mostly anogenital and oropharyngeal, where the virus provokes neoplastic transformation (10). HPV accounts for 4.5% of all human cancer globally (10). Besides cervical cancer (83% HPV related), HPV is found in cancer of the penis, vulva, vagina, anus, oropharynx (10).

The HPV-DNA test is approved by world health organization (WHO) as a primary screening tool for cervical cancer (11). Because HPV infection is predictive of future risk of cervical intraepithelial neoplasia (CIN) (12). High-risk HPV (in particular, type HPV-16 and HPV-18) persistent infection is by far the most important risk factor for high-grade disease progression, and these dysplastic lesions can remain stable, regress or progress and in severe cases, evolve into invasive cancer (13).

The objective of this research is to assess how common human papillomavirus (HPV) infection is and to identify its genotype distribution in cervical samples collected from women between the ages of 20 and 50 in Erbil, located in the Kurdistan Region of Iraq. The study places particular emphasis on cases involving cervicitis and atypical squamous cells of undetermined significance (ASC-US).

Methods

Patient Selection

A prospective study recruited 113 cervical smears from Erbil Maternity Teaching Hospital and Mufti Medical Laboratory (MML) in the 20-50 age group with ASC-US and cervicitis, evaluating them during the recording period from August 2024 - January 2025, in Erbil. Socio-demographic/risk factors data were collected. Cervical swabs were collected for genotyping for HPV. Specimens were obtained with a plastic cytobrush and placed in a 1.5 mL tube for transporting and HPV genotyping. The samples were stored at-20 °C in a freezer until DNA extraction. Laboratory procedures Cervical smears were screened for cervical lesions and HPV DNA by Papanicolaou staining and polymerase chain reaction (PCR), respectively.

Cervical Cytology Investigations

To assess cervical abnormalities, including intraepithelial lesions

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(SILs), cervical smears from each participant were stained using the standard papanicolaou method. The abnormalities were examined microscopically based on the morphological appearance of the cells and classified by a cytopathologist following the 2001 Bethesda classification system (14). The Pap test was conducted in all participants in the study. Gynecologists adhered to standard cervical sampling procedures, using a cytobrush inserted into the cervical canal and rotated five full turns (360°) to collect cells for cytological analysis. The collected samples were processed using conventional pap smear (directly onto a slide) according to standard protocols (14, 15).

HPV detection and typing

The genetic material of the human papillomavirus was detected through PCR to identify the presence and type of the virus in cervical specimens. DNA extraction from the samples was performed using a (SORB- AM DNA Extraction) high + low papilloma strip kit according to the manufacturer's manual.

RT-PCR:

The bosphore HPV Detection Kit v2, is a Real-Time PCR in-vitro diagnostic medical device. (Bosphore HPV Detection Kit v2) that is capable of qualitatively detecting human papillomavirus, which is consisted from the 15 most frequent HPV genotypes observed in human liquid samples (16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, 68) and 17 low risk HPV genotypes of (6, 7, 11, 13, 34, 40, 42, 43, 44, 55, 61, 62, 70, 73, 74, 81, 91). Portions of the L1, E1, E2, E6 and E7 genes are amplified and detection by fluorescence is performed through the FAM: Fluorescein Amidite (green fluorescent), HEX: Hexachloro-fluorescein (yellow-green fluorescent), TEXAS RED: Sulforhodamine 101 acid chloride (red dye), CY5:Cyanine dye 5 (far-red fluorescent). An internal control, directed at the detection of a human endogenic nucleic acid sequence derived from human genome (GAPDH), is included in the PCR Mix to monitor the nucleic acid extraction, the presence of PCR inhibition, and sampling or application errors.

Hybridization and developing

At this stage, these are a binding of PCR-amplified DNA fragments to sets of probes placed on nylon substrates. Probes for each of the types of HPV detected are covalently attached to the membrane, as well as a probe for the amplification control (GADPH or β -globine), a probe for the control of developing, and two or three lines, one black and one or two red, for controlling strip position and for interpreting of test results (see after linked diagram). A conjugate (streptavidin-peroxidase) will bind to a biotin label, which is attached to the DNA fragment that is

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being amplified during PCR and hybridized to the different probes. After addition of the substrate for the peroxidase (TMB), a blue precipitate will appear at hybridization. As a final test result, a band pattern is found, which is evaluated using a control strip.

Ethical considerations

Ethical approval for this study was granted by the College of Health Sciences at Hawler Medical University. The research was conducted in accordance with the ethical guidelines established by the university's ethics committee (Reference No. 2024 Sc.E.C. 10 G1)

Statistical analysis

Data was managed using SPSS version 26. The average and standard deviation were calculated in each group. Normality assumptions were tested, and a parametric approach was selected based on the results. A t-test for paired samples was utilized to compare methods at different follow-ups, and the Chi-square test was used for the comparison of categorical variables, as appropriate. For all analyses, P <0.05 was used for statistical significance (16).

Results

There were statistically significant association between cervicitis and ASCUS groups with different age groups using P-value 0.019. The majority of patients diagnosed with cervicitis were within the 35-39 age group, accounting for 26.3% of cases. In contrast, most patients with ASCUS were in the 30-34 age group, representing 29.7%, as shown in Table 1. This distribution highlights a notable age-related pattern in the prevalence of cervical lesions.

Table 1. Association between age groups and cervical abnormal cytology

Age	Group				
	Cervicitis No. (%)	Ascus No. (%)	Total No. (%)	Chi square	P-value
<30	13 (17.1)	9 (24.3)	22 (19.5)		
30-34	17 (22.4)	11 (29.7)	28 (28.8)		
35-39	20 (26.3)	1 (2.7)	21 (18.6)	11.759	0.019
40-44	10 (13.2)	6 (16.2)	16 (14.2)		
≥45	16 (21.1)	10 (27.0)	26 (23.0)		
Total	76 (100)	37 (100)	113 (100)		

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Cytology results

Out of the 113 cervical lesions, 76 patients (67.26%) diagnosed with Cervicitis while ASCUS cases were (32.74%) High percent of cervicitis cases were of mild category (23.89%) followed by severe cervicitis (23.01%) and moderate cervicitis (20.35%), respectively (Table 2, Figure 1).

HPV infection results showed that out of the 113 abnormal cervical samples, the percentage of patients with a positive PCR result (24.78%) And it was lower than that of patients with a negative PCR result (75.22%), most of them have high risk (21.24%) compared to low risk (3.54%). The percentage of patients with a single genotype result was 17 (15.04%) is higher than that of patients with a Multiple genotype were 11 (9.73%), (Figure 1).

Table 2 Descriptive statistics about abnormal cytology (N=113)

		N	%
Abnormal cytology	Cervicitis	76	67.26
	Ascus	37	32.74
Cervicitis category	Mild	27	23.89
	Moderate	23	20.35
	Severe	26	23.01

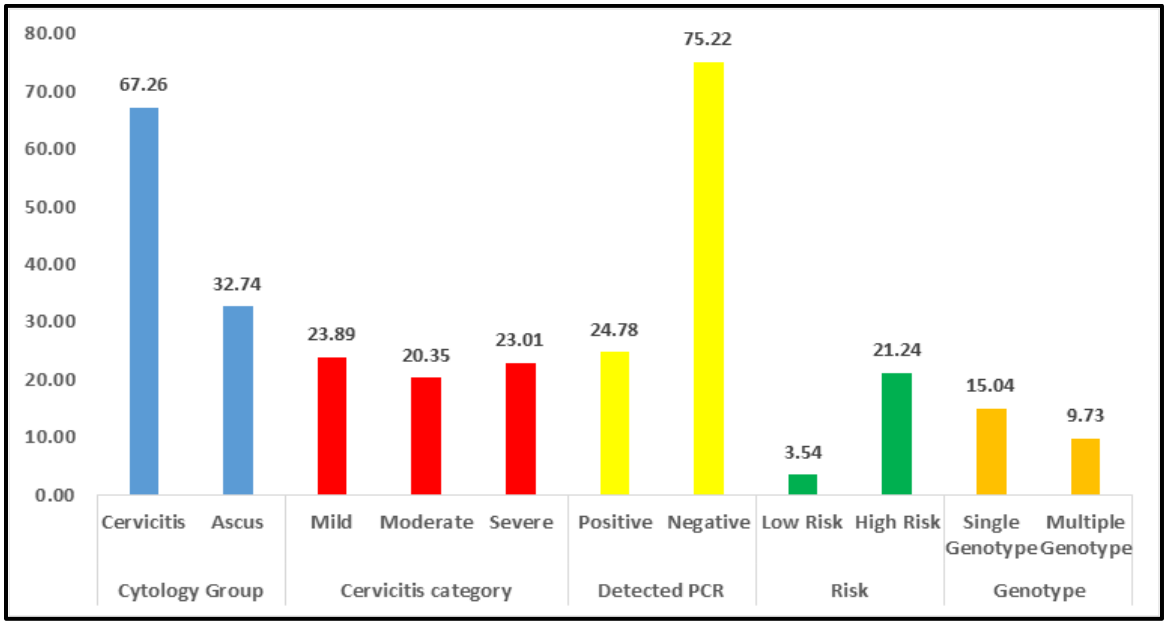

Figure 1. Percentages of (Cytology groups, Cervicitis category, Detected PCR results, Risk Factor, Genotype) using bar chart

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Association between abnormal cytology and HPV infection

Table 3 showed that there was statistically significant association between cervicitis and Ascus groups with PCR results-value 0.0001 Patients with positive ASCUS result (45.9%) is significantly higher compared to those with a positive cervicitis result (14.5%).

Out of the 17ASC-US cases, 5 with multiple HPV genotype infections, while 12 with single HPV genotype infections comprising five HPV-16 and one HPV-39, one HPV-42, one HPV-43, one HPV-58, one HPV-6, one HPV-68 and HPV-70. While out of the 11 Cervicitis cases 6 with multiple infection and 5 with single HPV genotype infection, two HPV-16, two HPV-51 and one HPV-68 showed there were no statistically significant association between cervicitis and ASC-US group with HPV genotype result P-value (0.175) (Table 4).

Table 3 Association between PCR group and Abnormal cytology

			Group				
			Cervicitis	**Ascus**	**Total**	**Chi square**	***P*-value**
Detected PCR	Positive	N	11	17	28		
		%	14.5	45.9	24.8	13.224	0.0001
	Negative	N	65	20	85		
		%	85.5	54.1	75.2		
Total		N	76	37	113		
		%	100.0	100.0	100.0		

Table 4. Association between genotype groups and abnormal cytology group

			Group		Total	Chi square	*P*-value
			Cervicitis	**Ascus**			
Genotype group	Single Genotype	N	5	12	17		
		%	45.5	70.6	60.7		
						1.783	0.175
	Combined Genotype	N	6	5	11		
		%	54.5	29.4	39.3		
Total		N	11	17	28		
		%	100.0	100.0	100.0		

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Table 5 showed statistically significant differences between the mean ages of patients with positive PCR results and those with negative PCR results (P-value of 0.007) specifically, the mean age of patients with positive PCR results is 33.45 years, which is notably lower than the mean age of patients with negative PCR results, at 38.06 years. This finding suggests a potential age-related trend in PCR outcomes, with younger individuals being more likely to test positive.

Moreover, the comparison of mean ages between patients with cervicitis and those presenting with ASC-US revealed no statistically significant difference (P = 0.923). There was no statistically significant difference between the mean ages of patients with low and high-risk results (P-value of 0.278).

Finally, there is no statistically significant difference between the mean ages of patients with single and combined genotype results (P-value of 0.998)

Table 5. Differences between age and each of the HPV, PCR, Risk, and Genotype groups using independent t-test

		N	Mean	Std. Deviation	t	P-value
HPV	Cervicitis	76	36.87	7.44	0.082	0.925
	Ascus	37	37.00	8.98		
PCR	Positive	28	33.43	7.20	3.754	0.007
	Negative	85	38.06	7.87		
Risk	Low Risk	4	29.75	2.63	1.108	0.278
	High Risk	24	34.04	7.57		
Genotype	Single	17	33.41	6.04	0.015	0.998
	Combined	11	33.45	9.04		

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Discussion

In our study all our cervical cases had abnormal cytological findings. The prevalence of ASCUS cytology among the participants in this study is moderately high (32.74%), with cervicitis having the highest prevalence (67.26%). The highest HPV prevalence in cervicitis cases was between ages 35-39 years (26.3%), while the prevalence decreases with increasing age. The same trend follows for ASCUS, with ages 30-34 years being the age group with the highest prevalence in both (29.7%) respectively. Consistent with our findings, a previous study from Iran reported that the highest prevalence of infection occurred among individuals aged 30 to 34 years, accounting for 151 cases, or 23.05% of the total (17). In the present study, the mean age among HPV-positive women in Erbil was 33.42 years. The most infected people fall between the age range of 30 and 34 years. (39.28%) total of 28 cases of patients. The above-mentioned results almost were consistent with the results of a research in Sanandaj-Iran with mean age (36.06 years) of women with HPV positive (16). Conversely, the prevalence of HPV was found to be low in samples of women over age 50 when compared with other age groups, and although the trend was toward a reduced prevalence as age increased, there were no women for whom the oldest attained age was 69 years (18). The overall prevalence of HPV infection in this study was 24.74%, with higher detection rate in the ASC-US group (45.9%) than cervicitis group (14.5%). This aligns with previous studies indicating a higher prevalence of HPV in women with ASC-US cytology results. For instance, a study in Duhok, Iraq, reported 40.6% HPV positivity rate among women by cytologic examination (Pap smear), with a higher frequency in ASC-US cases (19). Similarly, a study in Erbil by using polymerase chain reaction (PCR) found a 36% prevalence of HPV genotypes 16 and 18 among women with abnormal cervical cytology (20). In this study, HPV-16 was the most common genotype, detected in 25% of HPV-positive cases, HPV51 (6.19%), and (1.77%) respectively. The predominance of HPV-16 is consistent with global trends, as it is the most common high-risk HPV type associated with cervical cancer. These findings are similar to earlier studies, which demonstrated that the overall prevalence of high-risk HPV (HR-HPV) was higher than that of low-risk HPV (LR-HPV), and HPV-16 is the most common genotype (21). Our study found that 60.7% of HPV-positive cases had a single genotype infection, while 39.3% had multiple genotype infections. This distribution was similar to findings from other studies in the region; a study in Duhok reported that 6.25% of cases had mixed infections, primarily in ASC-US and low-grade squamous intraepithelial lesions (LSIL) (19). Other study reported a markedly

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different distribution, with 83.9% of HPV-positive cases showing single genotype infections, and only 16.1% showed multiple infections. This indicates that in that population, co-infection with multiple HPV types is significantly less frequent than in our area (22). A similar study in Tehran reported that the overall prevalence of human papillomavirus (HPV) was 61.2%. 36 (87.8%) women were infected with high-risk types of human papillomavirus, 26 (63.4%) with low-risk types and 2 (4.9%) with unknown types. The most common high-risk types were HPV 39 (43.9%), HPV 66 (31.7%), HPV 31 (26.8%) and HPV 18 (14.6%), respectively. HPV16 was detected in only 2 samples (4.9%) (23). The presence of multiple HPV genotypes in a significant proportion of cases underscores the complexity of HPV infections and the potential for co-infections to influence the progression of cervical lesions. On another hand, previous result reported that 13 out of 104 cervical samples (12.5%) tested positive for high-risk HPV genotypes. Among the positive cases, 4 out of 13 (30.7%) were identified as genotype 16, while 7 out of 13 (53.8%) showed mixed genotype infections (24).

Conclusion

The occurrence of both low and high-risk HPV genotypes were detectedamong patient with cervicitis and ASCUS.

HR-HPV16 is among the most common genotypes and had well known established strong association with cervical cancer. Therefore, it is highly recommended to develop new strategies for HPV-based cervical cancer screening, and the introduction of a prophylactic HPV vaccination program in Iraq is strongly advised.

Recommendations

Future studies with larger sample sizes and extended timelines would be crucial because such researches would provide more robust and statistically significant results, contributing to a deeper understanding of Human Papillomavirus genotyping among cervical patients in Kurdistan, Iraq. Additionally, comprehensive investigations could explore potential regional variations, risk factors, and the clinical implications of HPV infection in this specific population.

Competing interest

The author declares that he has no competing interests.

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