

Human Papillomavirus genotypes associated with cervical intraepithelial lesions among Saudi women

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Abstract. – **OBJECTIVE:** HPV is responsible for over 90% of cervical cancer worldwide. HPV has numerous subtypes with great variety distribution in different geographical regions. Thus, the present study aimed at assessing Human Papillomavirus (HPV) genotypes associated with cervical intraepithelial lesions among Saudi women.

PATIENTS AND METHODS: The current study collected cervical smears from 300 women transferred to a cytopathology laboratory between May 2020 and May 2021. The studied women were referred to do Pap smear because of gynecologic complaints.

RESULTS: Positive HPV testing was established in 4.7%. Most positive cases were identified in the age range of 14-50 years, followed by 51-60 years, representing 50% and 35.7%, respectively. The risk of HPV infection was significantly high in the age between 40-50 years, the odds ratio (OR), 95% confidence interval (95% CI), OR (95% CI) = 3.0137 (1.0228 to 8.8797), $p = 0.0454$, z statistic = 2.001. About 57% of the cases of HPV were found with cytological atypia. The risk of HPV in inducing cytological atypia, the OR (95%CI) = 1.8546 (2.9386 to 27.0952), $p = 0.0001$, z statistic = 3.862. Inflammatory cells infiltrate was identified in 43% of HPV-positive cases. The risk of HPV in inducing inflammatory changes, the OR (95% CI) = 2.6423 (0.8850 to 7.8887), $p = 0.0817$, z statistic = 1.741.

CONCLUSIONS: The prevalence of High-risk human papillomavirus is relatively low in the

AL-Madinah area, Saudi Arabia. Cervical cytological atypia corresponds to the positive HR-HPV findings. HR-HPV infection or cervical cytological atypical changes induce inflammatory cell infiltrates. HR-HPV infection is more common among elderly Saudi women.

Key Words:

Human papillomavirus, Cytological atypia, Cervical intraepithelial, Saudi Arabia.

Introduction

More than 99% of cervical cancer cases are caused by high-risk human papillomavirus (HR-HPV) subtypes, particularly HPV-subtypes 16 and 18. Worldwide cervical cancer is the fourth most common female cancer, strongly associated with spontaneous persistent genital infection with oncogenic HR-HPV. HR-HPVs usually infect the mucocutaneous epithelium, producing virions in mature epithelium cells, interrupting normal cell cycle control and promoting uncontrolled cell division, leading to the buildup of genetic mutations¹.

There are over 100 HPV subtypes responsible of multiple benign and malignant epithelial lesions². Subtypes can be divided in two groups:

HR-HPV subtypes, which include 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70; low risk HPV subtypes, comprising 6, 11, 42, and 44³.

However, there is a lack of literature regarding the epidemiology of HPV in Saudi Arabia. Some reports have revealed that the prevalence of HPV ranges from 4.5% to 5.9%, with the predominance of HPV-16 and HPV-18⁴⁻⁶. Although some studies have shown low prevalence rates of HPV-related cervical cancer in Saudi Arabia⁷, there is still scarce effort regarding the introduction of cervical early detection (Pap test) or prevention (vaccination). The initial step is to identify the most common HPV subtypes to prepare vaccine protocol. Therefore, the present study aimed to assess Human Papillomavirus genotypes associated with cervical intraepithelial lesions among Saudi women.

Patients and Methods

The current study collected cervical smears from 300 women transferred to a cytopathology laboratory at the maternity hospital in Al-Madi-

nah, Saudi Arabia, between May 2020 and May 2021. The studied women were referred to do Pap smear because of gynecologic complaints. A Pap smear is requested as a part of the required investigations in correlation with clinical examination. Before sample collection, each patient was asked to sign a written ethical consent form.

Cytological samples were collected by scraping the transformation zone of the ectocervix, fixed in 95% ethyl alcohol for 15 minutes, then stained according to the Papanicolaou method (Pap. Method) as described elsewhere⁸.

Cervical samples were further screened for HPV by molecular methods Polymerase Chain Reaction (PCR), adopting the procedure described by Golfetto et al⁹ (Table I).

Statistical Analysis

Data were initially arranged in an Excel sheet then transferred to SPSS software for analysis (IBM Corp., Armonk, NY, USA) to obtain frequencies, percentages, means, and cross-tabulations, and the Chi-square test. The *p*-value < 0.05 is considered statistically significant contemplating 95% confidence interval.

Table I. Cervical samples screened for HPV by molecular methods Polymerase Chain Reaction (PCR).

HPV Genotype	Sequence (5'-3')	Amplification (bp)
16	5'-CAC AGT TAT GCA CAG AGC TGC-3' 5'-CAT ATA TTC ATG CAA TGT AGG TGTA-3'	325
18	5'-CAC TTC ACT GCA AGA CAT AGA-3' 5'-GTT GTG AAA TCG TCG TTT TTC A-3'	425
31	5'-GAA ATT GCA TGA ACT AAG CTC G-3' 5'-CAC ATA TAC CTT TGT TTG TCA A-3'	520
33	5'-ACT ATA CAC AAC ATT GAA CTA-3' 5'-GTT TTT ACA CGT CAC AGT GCA-3'	227
35	5'-CAA CGA GGT AGA AGA AAG CAT C-3' 5'-CCG ACC TGT CCA CCG TCC ACCG-3'	280
39	5'-GAC GAC CAC TAC AGC AAA CC-3' 5'-TTA TGA AAT CTT CGT TTG CT-3'	340
45	5'-TAA ACA GTT ATA TGT AGT GTA CCG-3' 5'-TAT CAG CAC GTC CAG AAT TGA C-3'	475
51	5'-GAG TAT AGA CGT TAT AGC AGG-3' 5'-TTT CGT TAC GTT GTC GTG TAC G-3'	223
52	5'-TAA GGC TGC AGT GTG TGC AG-3' 5'-CTA ATA GTT ATT TCA CTT AAT GGT-3'	360
56	5'-GTG TGC AGA GTA TGT TTA TTG-3' 5'-TTT CTG TCA CAA TGC AAT TGC-3'	325
58	5'-GTA AAG TGT GCT TAC GAT TGC-3' 5'-GTT GTT ACA GGT TAC ACT TGT-3'	240
59	5'-CAA AGG GGA ACT GCA AGA AAG-3' 5'-TAT AAC AGC GTA TCA GCA GC-3'	395
66	Sense: TTC AGT GTA TGG GGC AAC AT AAA CAT GAC CCG GTC CAT GC	304

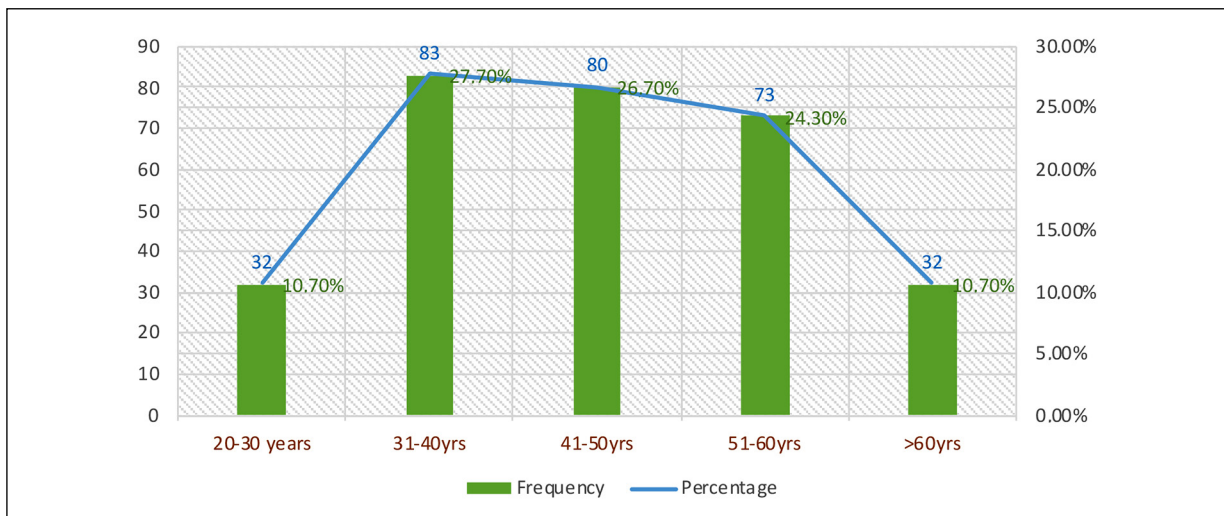


Figure 1. Description of the study subjects by age.

Results

In this study, we investigated cervical smears from 300 women for the presence of HPV aged 20 to 70 years were cytologically investigated. Most women were aged 31-40 years followed by 41-50, 51-60, 20-30, and > 60 years, representing 27.7%, 26.7%, 24.3%, 10.7%, and 10.7%, respectively, as shown in Figure 1.

Positive HPV testing was established in 14/300 (4.7%). Most positive cases were identified in the age range 14-50 years, followed by 51-60 years, representing 7/14 (50%) and 5/14 (35.7%), respectively. The risk of HPV infection was significant-

ly high in the age between 40-50 years, the odds ratio (OR), 95% confidence interval (95%CI), OR (95%CI) = 3.0137 (1.0228 to 8.8797), $p = 0.0454$, z statistic = 2.001.

About 8/14 (57%) of the cases of HPV were found with cytological atypia. The risk of HPV in inducing cytological atypia, the OR (95% CI) = 1.8546 (2.9386 to 27.0952), $p = 0.0001$, z statistic = 3.862.

Inflammatory cells infiltrates were identified in 6/14 (43%) of HPV positive cases. The risk of HPV in inducing inflammatory changes, the OR (95% CI) = 2.6423 (0.8850 to 7.8887), $p = 0.0817$, z statistic = 1.741, as, indicated in Table II, Figure 2.

Table II. Distribution of HPV infection by age and cytopathological changes.

Variable	HPV-Positive	HPV-Negative	Total
Age			
20-30 years	0	32	32
31-40	1	82	83
41-50	7	73	80
51-60	5	68	73
> 60	1	31	32
Total	14	286	300
Cytological changes			
Mild atypia	3	7	10
Medium Atypia	3	8	11
Severe atypia	2	10	12
Normal	6	261	267
Total	14	286	300
Inflammatory cells infiltrate			
Yes	6	65	71
No	8	221	229
Total	14	286	300

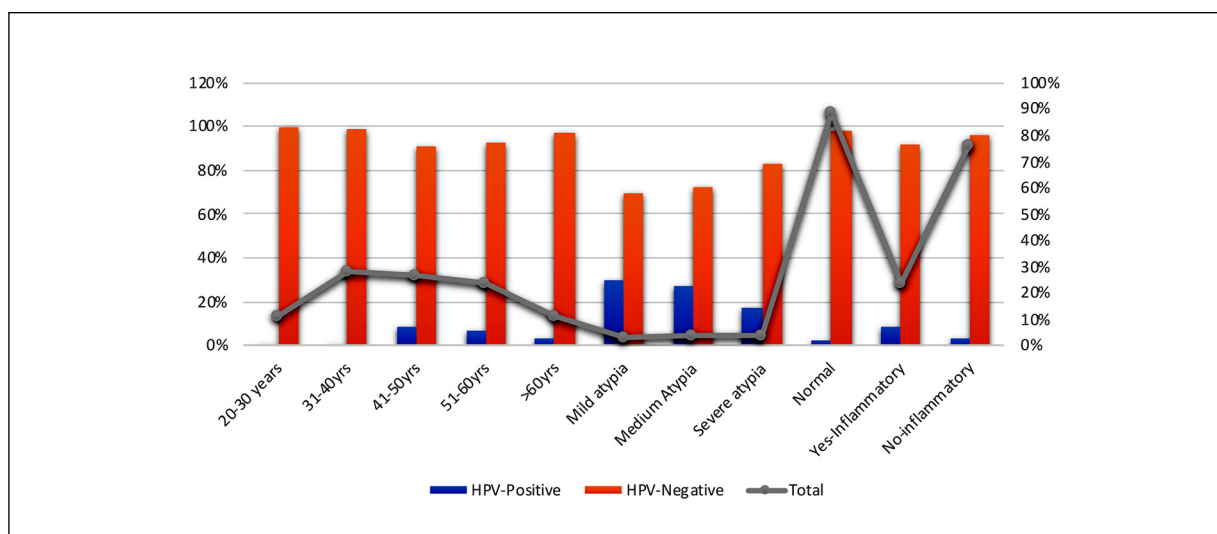


Figure 2. Description of the HPV infection by cytological changes.

Table III, Figure 3 described the summarizing of HPV subtypes by age and cytological changes. Most patients in this series were infected with HPV-16, followed by HPV-52, HPV-58, and HPV-33, constituting 6/14 (42.9%), 3/14 (21.4%), 2/14 (14.3%), and 2/14 (14.3%), in this order.

Of the seven positive cases in the age group 41-50 years, 5/7 (71.4%) were HPV-16.

Of the five positive cases in the age group 51-60 years, 2/5 (40%) were HPV-52, and 2/5 (40%) were HPV-33. Mild atypia was associated with one case in HPV-18, HPV-16, and HPV-58 positive cases. Of the 3-moderate atypia, 2/3 (66.7%)

were positive with HPV-33. One case of severe atypia was associated with HPV-16, and the other was HPV-52. Most inflammatory cells infiltration 3/6 (50%) was associated with HPV-16.

Discussion

In the present study, the overall prevalence of HPV in these series of patients was 4.7%, which is lower than the previously reported⁵ prevalence rates in Saudi Arabia. In fact, in 2013, a prevalence rate of 5.6% of HR-HPV among women

Table III. Distribution of the HPV subtypes by age and cytological changes.

Variable	HPV-Subtypes					Total
	HPV16	HPV18	HPV52	HPV58	HPV33	
Age						
31-40	0	0	1	0	0	1
41-50	5	1	0	1	0	7
51-60	0	0	2	1	2	5
> 60	1	0	0	0	0	1
Total	6	1	3	2	2	14
Cytopathology						
Mild atypia	1	1	0	1	0	3
Moderate Atypia	1	0	0	0	2	3
Severe atypia	1	0	1	0	0	2
Normal	3	0	2	1	0	6
Total	6	1	3	2	2	14
Inflammatory changes						
Yes	3	1	1	0	1	6
No	3	0	2	2	1	8
Total	6	1	3	2	2	14

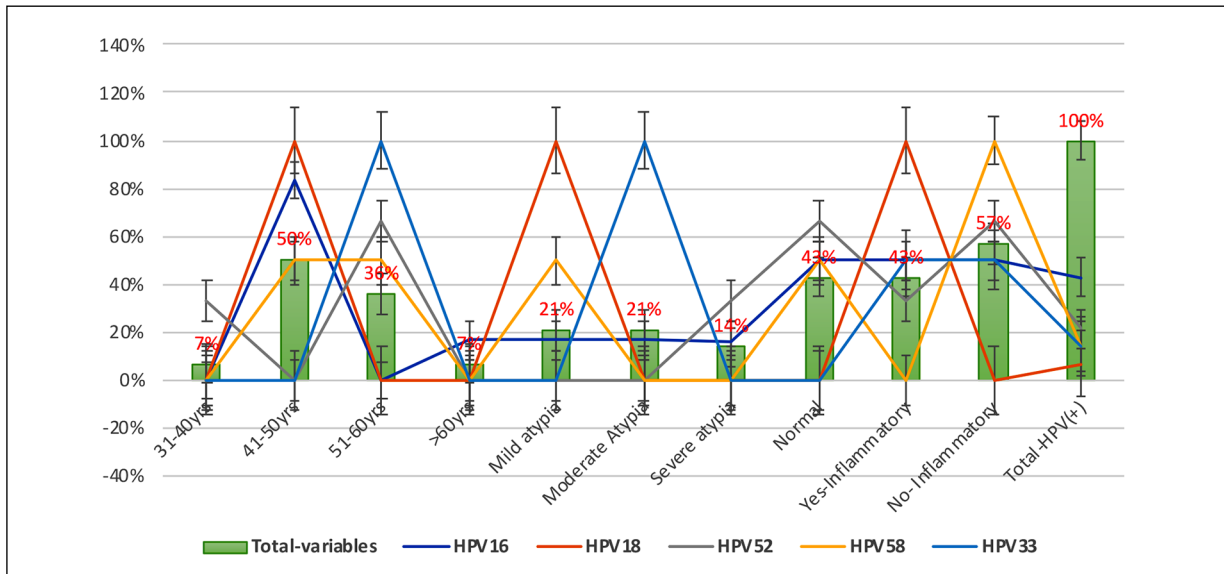


Figure 3. HPV subtypes by age and cytological changes.

pursuing gynecologic care was reported in Western Saudi Arabia. In 2014, a study¹⁰ from Riyadh, Saudi Arabia, reported a high prevalence rate of HPV (31.6%). Another survey from Western Saudi Arabia has reported a rate of 5.9% in 2019.

The most frequently reported HPV subtypes in this study were HPV-16, followed by HPV-52, HPV-58, and HPV-33, comprising 42.9%, 21.4%, 14.3%, and 14.3%, respectively. A recent study¹¹ from Eastern Saudi Arabia investigated 164 women and revealed an HPV prevalence of 14.5%, with the most frequent HPV subtype 16 (33.3%), HPV16&18/45 (8.3%), while the remaining (58.3%) scattered in different HR-HPV subtypes. Another Saudi study¹² has reported that, irrespective of solo or manifold infections for every case, HPV-16 was discovered in 87.8%, followed by HPV-18 in 86%, and HPV-11 identified in 78.3%. However, it is well established that HR-HPV 16/18 is responsible for about 70% of cases of cervical cancer¹³.

Most cases with positive HPV were found in the age range 41-60 years. The age of women suspected of getting an infection or persistent infection varies greatly. This is determined by several factors¹⁴. In Saudi Arabia, the peak incidence of cervical cancer is at around 43 years, with a slighter jump back at approximately 61 years¹⁵.

In the current study, about 57% of the cases of HPV were found with cytological atypia. The risk of HPV in inducing cytological atypia, the OR (95%CI) = 1.8546 (2.9386 to 27.0952), $p = 0.0001$, z statistic = 3.862. Many studies¹⁶ have found

a correlation between cervical cytology atypical changes and HPV infection. However, there is a running debate whether cytology can determine HPV infection status or if HPV testing can replace Pap. Smear screening. A study¹⁷ investigated the disagreements between cervical cytological atypia and HR-HPV status. Several cases of HPV infection but with normal cytology were found. When there are discrepancies in this context, a careful HPV status and the degree of cytological atypia should be done before further mediation. However, the rate of cervical cancer among HPV 16/18-infected women with negative cytology is the same as women with atypical cytology¹⁸.

In the current study, inflammatory cells infiltration was established in 43% of patients with positive HPV. The presence of HPV or cytological atypia was a substantial factor influencing the production of numerous inflammatory mediators and inflammatory cells in the cervix¹⁹.

Conclusions

The prevalence of high-risk Human Papillomavirus is relatively low in the Al-Madinah area, in Saudi Arabia. Cervical cytological atypia corresponds to the positive HR-HPV findings. HR-HPV infection or cervical cytological atypical changes induce inflammatory cells infiltration. HR-HPV infection is more common among elderly Saudi women.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

The authors would like to thank Dr. Hashim M. Missawi at the Department of clinical pathology, maternity, and children's hospital, for his help in the sample collection and consultation.

Ethical Approval

The Ethical Committee approved the present study's proposal at the College of Medicine, University Ha'il, Saudi Arabia (HREC 00130/CM-UOH.04/20).

Informed Consent

Each participant was asked to sign a written ethical consent before the interview.

Availability of Data and Materials

All data and materials were included in the article.

Funding

This research has been funded by the Scientific Research Deanship at the University of Ha'il, Saudi Arabia, through the project number RG-20193.

Authors' Contribution

FDA: Conception, administration, analysis, drafting, approval of the final version. SAA: Conception, design, data acquisition, practical part, approval of the final version. MIH: Conception, analysis, drafting, practical part, approval of the final version. EKA: Conception, design, data acquisition, approval of the final version. MABB: Conception, analysis, drafting, approval of the final version. AEO: Conception, analysis, drafting, approval of the final version. AAA: Conception, analysis, drafting, approval of the final version.

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