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Coinfection of HPV18 with some high-risk less frequent HPV subtypes

Musa Abdalla Ali[1*], **Saleh Hussein Bensumaidea**[2], **Hussain Gadelkarim Ahmed**[1]

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Authors' Affiliation:
[1]Faculty of Medical Laboratory Science, University of Khartoum, Sudan
[2]Department of Pathology, College of Medicine, Hadhramout University of Science and Technology, Yemen

[*]**Corresponding Author**
Faculty of Medical Laboratory Science, University of Khartoum, Sudan
Email: dr.musa@yahoo.com

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ABSTRACT

Background: High-Risk Human Papillomavirus (HR-HPV) is a major health concern worldwide. Thus, this study aimed to assess the coinfection of HPV18 with some high risk less frequent HPV genotypes including HPV subtypes 31, 33, 35, 39, 45, 52, 56, 58, 59 and 66. *Methodology:* This is a retrospective study of 200 cervical samples retrieved from Yemeni histopathological laboratories exist in Hadhramout, Yemen. All samples were formalin-fixed paraffin wax-processed tissues. All specimens had a previous diagnosis of cervical cancer by conventional histopathology. *Results:* The most common pathological condition was squamous cell carcinoma (SCC), followed by adenocarcinoma, CIN3, and CIN1, accounting for 53.3%, 12.7%, 10.7%, and 10%, respectively. Coinfection with HPV 18 was detected in 12.5% with 3% with HPV 31, HPV35, and 3% with HPV58. *Conclusion:* Infection with HPV is widespread and the causes of increased cases of cervical cancer. The most common genotypes of HPV that coinfect with HPV subtype 18 are HPV31, HPV35, and HPV58.

Keywords: Human Papillomavirus, Coinfection, Cervical cancer, Yemen, HR-HPV

1. INTRODUCTION

The Human Papillomavirus (HPV) is the highest prevalent sexually transmitted viral infection worldwide. High-Risk Human Papillomavirus (HR-HPV) subtypes are responsible for over 99% of cervical cancers. The prevalence of HPV is strongly influenced by geographical burden (Scott-Wittenborn and Fakhry, 2021). Varied disparities in cervical cancer epidemiology and mortality rates were noted in numerous geographical areas. The highest incidence rates were observed in Sub-Saharan Africa. About 85% of the deaths are encountered in developing regions of the world. Persistent infection with HR-HPV genotypes represents the major concern about cervical cancer burden in these regions. HR-HPV subtypes 16, 18, 31, 58, and 52 are rendered the most common genotypes associated with cervical cancer worldwide. These HPV subtypes are responsible for over 50% of all HPV infections with HR-HPVs 16 and 18 infections reporting for approximately 70% of the whole infection (Pimple and Mishra, 2022; Barhamain et al., 2022).

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HR-HPV subtypes include 16,18,31,33,45,35,52,56,39,51,68, and 59. Moreover, HPV 53, 66, 26, 73, and 82 are regarded as potential HR-HPV subtypes (Zhong, et al., 2022). World Health Organization (WHO) has reported that cervical cancer represented 0.08% of all deaths in Yemen (World health Ranking, 2020). Studies from Yemen have shown that the common HPV subtypes are HPV 16 and HPV 18 with prevalence rates of HPV-16 being 37% and HPV18 being 16% (Ahmed et al., 2017; Ahmed et al., 2015). Several studies have well documented that more than one HPV genotype can infect one woman (Chaturvedi et al., 2011; Kim et al., 2021). Therefore, the present study aimed to assess the coinfection of HPV18 with some high risk less frequent HPV subtypes.

2. MATERIALS AND METHODS

This retrospective investigation included, 200 cervical samples were retrieved from Yemeni histopathological laboratories exist in Hadhramout, Yemen. All samples were formalin-fixed paraffin wax-processed tissues. All samples were previously diagnosed with cervical cancer using conventional histopathology. The study was conducted from June 1st 2022 to June 23/2022.

Immunohistochemistry

Thin tissue sections were obtained and immunologically stained applying P16 ^{INK4a} antibody adopting the Avidin-Biotin method, as described elsewhere (Liu et al., 2017)

Polymerase chain reaction (PCR)

DNA was extracted from the thin sections obtained from the tissue blocks. All quality control measures were strictly followed during molecular analysis. PCR was done by adopting the procedure described elsewhere (Ahmed et al., 2017).

Ethical consent

The protocol of the current study was approved by Human Research Ethics Committee (HREC) at the Faculty of Medical Laboratory Science board, University of Khartoum, Sudan. Approval number: HREC 0001/FMLS-UOK.6/22.

Statistical analysis

Collected data was set in standard sheets and entered computer software (SPSS) and analyzed to obtain frequencies, cross-tabulations, relative risk, and Chi-square test considering a 95% confidence interval. A P-value below 0.05 was identified as statistically significant.

3. RESULTS

This study investigated 200 women, aged 22 to 75 years old with a mean age of 47 years. Out of the 200 women, 50/200(25%) were found with no apparent lesions. The most frequent pathological condition was squamous cell carcinoma (SCC), followed by adenocarcinoma, CIN3, and CIN1, representing 80/150(53.3%), 19/150(12.7%), 16/150(10.7%), and 15/150(10%), in that order. Most pathological conditions were noted in the age group 46-55 years followed by 36-45, and 56+ years, constituting 55/150(36.7%), 46/150(30.7%), and 27/150(18%), respectively, as indicated in Table 1, Fig 1. However, percentages with entire pathological groups show some variations, as indicated in Table 1, Fig 2.

Table 1 Distribution of the study subjects by age and pathology

Variable	<35 years	36-45	46-55	56+	Total
CIN1	3	7	3	2	15
CIN2	3	1	6	3	13
CIN3	2	7	5	2	16
SCC	12	23	30	15	80
Adenocarcinoma	2	4	8	5	19
Adenosquamous Ca	0	2	1	0	3
Clear cell Ca	0	1	1	0	2
Glassy cell Ca	0	1	1	0	2
No cervical lesions	9	14	17	10	50
Total	31	60	72	37	200

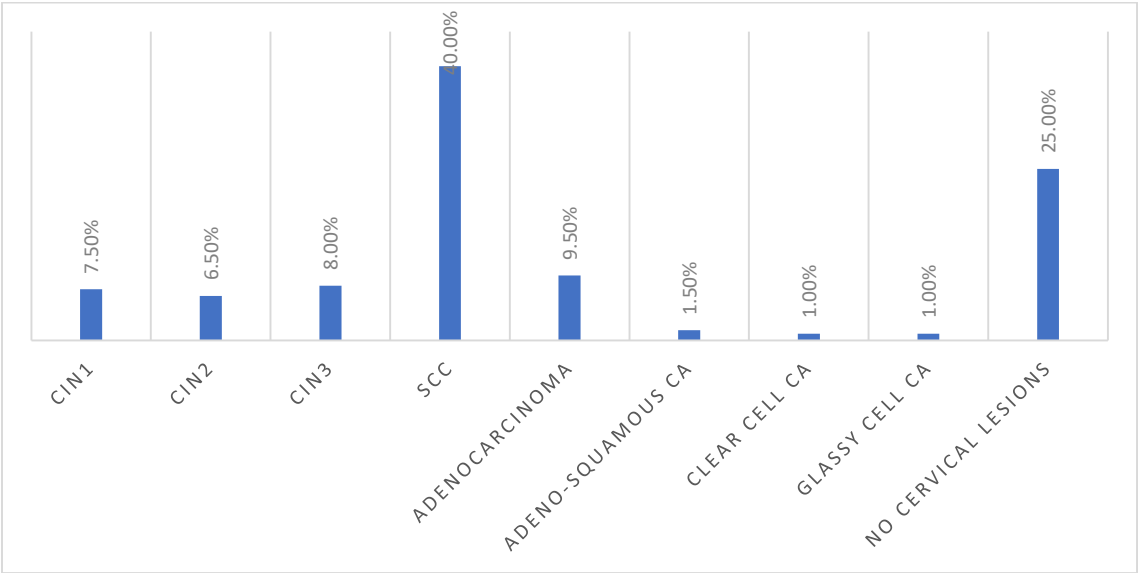

Figure 1 Description of the study population by pathology

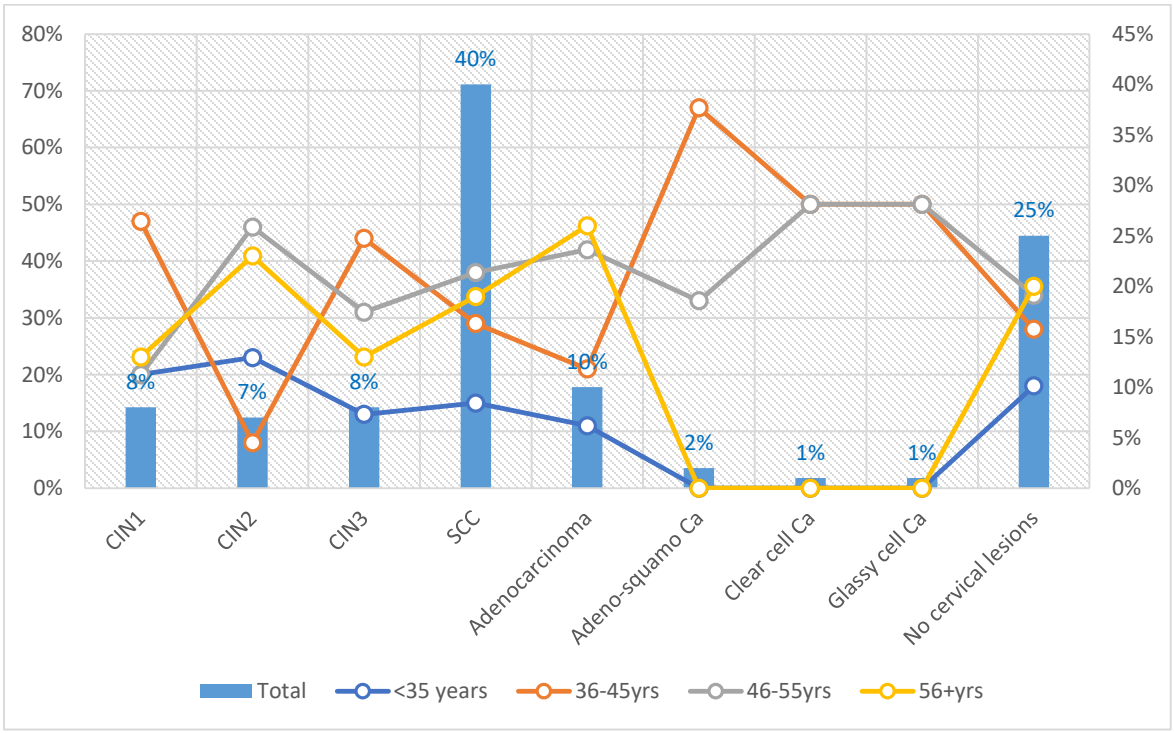

Figure 2 Description of the age in entre pathological condition types.

Out of 200 patients, HR-HPV immunohistochemical test has revealed infection in 84/200(42%). Most positive cases were seen among SCC followed by CIN3, and adenocarcinoma, representing 45/84(53.5%), 10/84(11.9%), and 8/84(9.5%), respectively (Table 2, Fig 3).

Table 2 Distribution of the pathology by HR-HPV applying P16 antibody

Variable	HR-HPV by P16 antibody		
	Positive	Negative	Total
CIN1	4	11	15
CIN2	7	6	13
CIN3	10	6	16

SCC	45	35	80
Adenocarcinoma	8	11	19
Adenosquamous Ca	3	0	3
Clear cell Ca	1	1	2
Glassy cell Ca	0	2	2
No cervical lesions	6	44	50
Total	84	116	200

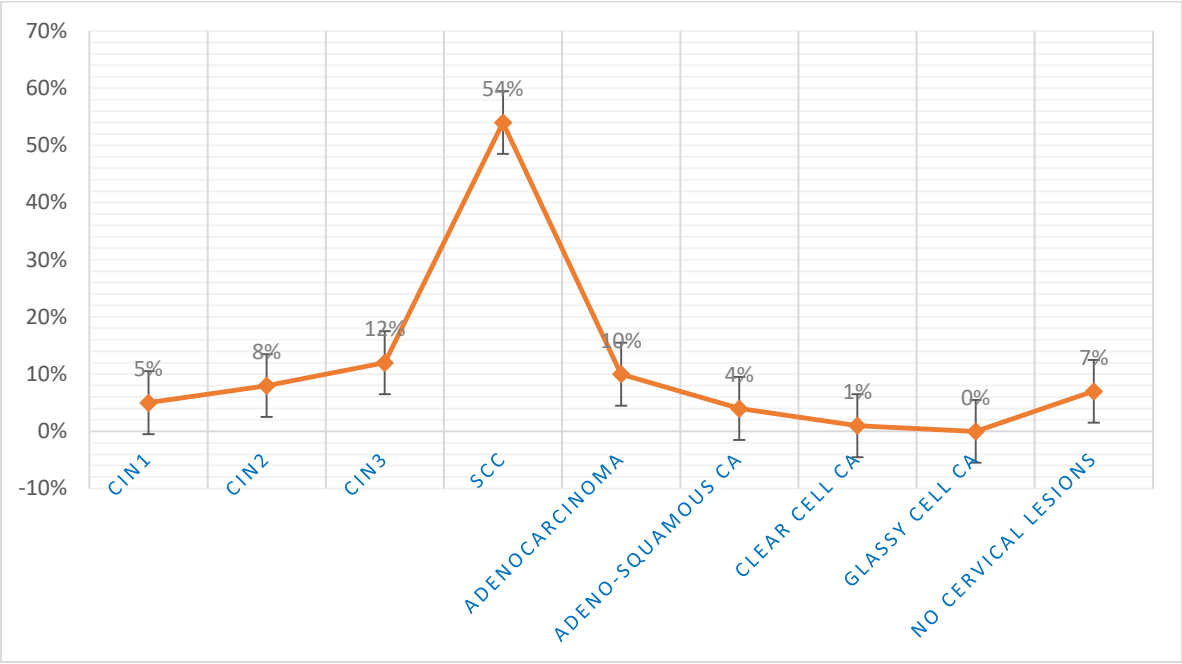

Figure 3 Description of pathology by positive HR-HPV (P16 antibody).

Table 4 summarized the distribution of HR-HPV 18 by coinfection with HPV subtypes 31, 33, 35, 39, 45, 52, 56, 58, 59 and 66. For HPV31, coinfection with HPV18 was noted in 4/32(12.5%) cases. The risk of HPV31 coinfection with HPV 18, the relative risk (RR) and 95% confidence interval (95%CI) was RR(CI95%) = 0.8571(0.3829 to 1.9188), P = 0.7077, z statistics = 0. 375. Positive coinfection was noted with HPV35 and HPV58 in one case 1/32(3%) for each.

Table 4 Distribution of HR-HPV 18 by coinfection with HPV subtypes 31, 33, 35, 39, 45, 52, 56, 58, 59 and 66.

Variable	HR-HPV 18		
	Positive	Negative	Total
HPV31			
Positive	4	6	10
Negative	28	162	190
Total	32	168	200
HPV33			
Positive	0	6	6
Negative	32	162	194
Total	32	168	200
HPV35			
Positive	1	5	6
Negative	31	163	194
Total	32	168	200
HPV39			
Positive	0	5	5
Negative	32	163	195

Total	32	168	200
HPV45			
Positive	0	10	10
Negative	32	158	190
Total	32	168	200
HPV52			
Positive	0	1	1
Negative	32	167	199
Total	32	168	200
HPV56			
Positive	0	0	0
Negative	32	168	200
Total	32	168	200
HPV58			
Positive	1	6	7
Negative	31	162	193
Total	32	168	200
HPV59			
Positive	0	4	4
Negative	32	164	196
Total	32	168	200
HPV66			
Positive	0	0	0
Negative	32	168	200
Total	32	168	200

4. DISCUSSION

There is a paucity of data from Yemen regarding HPV and even cervical cancer. Therefore, the present study was aiming to assess the coinfection of HPV18 with some high risk less frequent HPV genotypes including HPV subtypes 31, 33, 35, 39, 45, 52, 56, 58, 59 and 66. However, high rates of HPV 18 coinfections were identified with HPV-31. HPV18 and HPV31 were categorized under the 5 most common HPV genotypes that affect women worldwide (Pimple and Mishra, 2022). Moreover, HPV18 and HPV16 are the most potent strains that are responsible for over 70% of the cases of cervical and head, and neck cancers worldwide (Yu et al., 2022). The most potent oncogenes associated with HPV 18 are *E6*/*E7* (Tian et al., 2022).

HPV31 is relatively similar to the most carcinogenic HPV16, though it was proposed to be responsible for only 4% of the cases of cervical cancer. Although still little is known about HPV31, its genetic and epigenetic variation has been linked to the carcinogenesis of other HR-HPV genotypes (Pinheiro et al., 2021). A study that looked at the whole-genome methylation patterns of HPV genotypes 31, 18, and 45 discovered that there was a strong increased degree of methylation at several CpG sites in E2, L2, and L1 regions for all three genotypes. The highest areas under the curve were 0.81 for HPV31, 0.85 for HPV18, and 0.98 for HPV45. These results suggest that multiple infections can happen, and methylation can reveal which of the infections are causal (Wentzensen et al., 2012).

Coinfection of HPV18 was also revealed with HPV type 35. HPV35 is responsible for about 2% of invasive cervical cancers worldwide and around 10% in Sub-Saharan Africa (Pinheiro et al., 2020). A study assessed the epidemiology of HPV genotypes in cervical cancer in South Africa and found that HPV 35 was the third most common genotype with cervical precancerous lesion 12.6%; single infection: 5.7% and multiple infections: 6.9%. The study has proposed that HPV35 warrants attention, and it should be included in the available vaccines (Mbulawa et al., 2022). Although HPV 35 is not a commonly identified HR-HPV subtype in North America and Europe, it was reported as the most common high-risk type in a cohort in rural Zimbabwe (Megan et al., 2020).

Coinfection of HPV18 was also discovered with HPV type 58. HPV58 can be encountered in precancerous and cancerous cervical lesions. When compared to cervical lesions of lesser severity, the HPV58 E2/E6 copy number ratio in patients with cervical cancer is lower, indicating a high level of viral integration. This may suggest a significant risk factor for cervical cancer (Jianhua et al., 2017). Although there is a lack of data regarding HPV coinfection as well as the scarcity of data from Yemen, Reduced rates of

HPV infection were noticed in the Middle East, which may be due to the cultural norms and conservative sexual behaviors (Pimple and Mishra, 2022).

5. CONCLUSION

Infection with HPV is prevalent and responsible for increased cases of cervical cancer. The most common HPV genotypes coinfected with HPV-subtype 18 are HPV31, HPV35, and HPV58. Research on HPV is urgently needed to facilitate the introduction of an appropriate vaccine in the country.

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Informed consent

Written & Oral informed consent was obtained from the patient identified in this study.

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This study has not received any external funding.

Conflicts of interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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