Received: February 21, 2024
Accepted: April 11, 2024

Acad Med J 2024;4(1):30-40
UDC:618.146-006.6-022.6:578.827
https://www.doi.org/10.53582/AMJ2441030kk
Original article

HUMAN PAPILLOMA VIRUS INFECTIONS, VIRAL LOAD AND AGE IN WOMEN WITH CERVICAL CANCER

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Abstract

Introduction: Persistent infection with high-risk human papillomavirus (HPV) is the primary cause of preinvasive and invasive cervical lesions. The study aimed to investigate associations between HPV infections, age, HPV DNA viral load, and cytological abnormalities.

Material and methods: A total of 300 women, aged 18-65, were enrolled in this study as part of a primary cervical cancer screening program, from February 1st 2019 to August 30th 2023. Participants were referred to the Microbiological Laboratory of the Bitola Public Health Center for HPV genotyping, conducted concurrently with the Pap test. Specimens for HPV genotyping were collected by cervical canal scraping. DNA extraction used the genomic DNA Kit by Life-Technology. HPV genotypes and viral DNA concentration were determined using the HPV Quantitative Real-time PCR Kit, DTlite, Russia.

Results: Participants were categorized into a control group, 97 patients with normal Pap tests, and a study group (119 with low-risk cervical lesions - non-hrCL, and 87 with high-risk cervical lesions - hr-CL). HPV infections exhibited a bimodal distribution, with peaks in women aged 18-34 and 55-64. MT-HPV infection correlated with a higher proportion of hr-CL (54.76%) compared to non-hrCL (41.18%) and normal findings (31.96%). Viral loads for specific HPV types increased linearly with worsening cervical lesions, except for HPV-18, -45, -56, and -59, which exhibited a decrease in viral loads.

Conclusion: Associations between type-specific viral load and cervical pathology severity underscore the significance of enhanced measures for treating and monitoring patients infected with high-risk HPV, particularly those with MT-HPV infections and high viral loads.

Keywords: human papillomavirus, multi-type HPV infection, age, HPV DNA viral load

Introduction

The most prevalent sexually transmitted infection in both the United States and Europe is the infection caused by the human papillomavirus (HPV)[1]. The primary factor leading to precancerous lesions and cervical cancer is regarded as the persistent infection with a high-risk genotype of the HPV[2]. Cervical carcinoma and other HPV-related cancers represent a significant global public health problem. Cervical cancer is the fourth most common malignant tumor in women worldwide. According to 2020 Globocan study, there are approximately 604,127 new cases and 342,000 deaths associated with cervical cancer annually[3].

According to 2020 data from the Information Center for HPV, established by the Catalan Institute of Oncology and the International Agency for Research on Cancer (ICO/IARC), 113 new cases of cervical cancer were recorded in the Republic of North Macedonia (RNM). The data reveals that cervical cancer ranks as the fifth most common malignant disease among all women in the RNM and the third most frequent among women aged 15 to 44 years[4].

A total of 225 HPV genotypes have been identified to date, 40 of them being sexually transmitted. Based on their oncogenic potential, the International Agency for Research on Cancer (IARC) in 2003 divided these genotypes into three groups[5]. The first group comprises 14 high-risk HPV types (HR-HPV), including HPV16, -18, -31, -33, the second group includes 6 potentially high-risk types of HPV, and the third group contains 31 low-risk HPV types (LR-HPV), including HPV 6, -7, -11[6]. HPV DNA of high-risk genotypes is detected in 99.7% of cervical cancer tissues, and persistent infection with HR-HPV types, especially HPV16 and HPV18, is recognized as a key factor in the development of cervical cancer[7-9]. Cervical carcinoma develops as a result of frequent acute HPV infections, less frequently recognized persistent HR-HPV infections, and a series of stages histologically classified as cervical intraepithelial neoplasia 1 to 3 (CIN1 to CIN3), or cytologically defined as a squamous intraepithelial lesion (SIL). HPV infection can cause a low-grade squamous intraepithelial lesion (LSIL), and a high-grade squamous intraepithelial lesion (HSIL)[10].

Several factors are believed to contribute to persistent HPV infection. These factors include viral characteristics such as HPV genotype, infections caused by single type (ST) or by multiple types (MT) of HPV[11,12], HPV DNA viral load[13-15], as well as host factors like age[15-16], immunosuppression, genetic predisposition, and environmental factors such as smoking[17] and sexual behavior[18]. Understanding these intricate correlations is crucial for early detection and prevention of cervical cancer through effective screening and vaccination programs.

In the literature, there is a lively discussion about the diagnostic value of identifying factors that influence on persistent infection, such as ST-HPV or MT- HPV infections, the age of patients and HPV DNA viral load.

Aims

To investigate the correlation between infections caused by single or multiple types of HPV and the age of patients.

To examine the prevalence of infections caused by ST-HPV or MT-HPV in relation to the cytological findings of patients.

To study the correlation of HPV DNA viral load with the cytological findings of patients.

Material and methods

The study has a retrospective-prospective, cross-sectional, single-point design.

3.1.0 Study population

The study comprised 300 women, aged 18-64 from Bitola, Resen, and Kichevo, who voluntarily visited their family gynecologist between February 2019 and August 2023, as part of the primary cervical screening program. During the visit to the gynecologist, patients had a complete physical examination, and two swabs were taken, one for a Pap test and the second one for a HPV DNA testing. After the examination, the women were referred to the Center for Public Health Bitola, for molecular analysis of the sample.

The study was conducted at the Public Health Center Bitola, in the Department of Microbiology, specifically Department of Serology with Molecular Microbiology, where

molecular tests for detection of HPV genotypes were performed and concentration of HPV DNA in the samples was determined. Pap smears were analyzed by a pathologist, and data were obtained in collaboration with family gynecologists.

The study participants were categorized into two groups: control group and study group. The control group included patients whose cytological smear was negative during the study and had no history of an abnormal Pap test, but had a positive HPV detection test. The study group included patients who had an abnormal Pap test and a positive test for detection of the examined types of HPV.

The study's inclusion criteria were women aged 18 to 65 years who provided signed consent, were not pregnant, had no history of surgical procedures like hysterectomy, and had not undergone cervical treatment in the last 6 months. Exclusion criteria were: women lacking complete HPV or Pap test results, insufficient sample sizes, a history of gynecological cancers, and the presence of chronic diseases such as liver cirrhosis, renal failure, or cardiovascular disease.

3.2.0 Sampling procedure

During a comprehensive cervical examination, the gynecologist obtained two exfoliative cervical samples from each participant. The initial sample collected by using a cytobrush, a specialized brush for cervical cells, was employed in the preparation of a conventional Pap test. Subsequently, a second cervical sample, acquired with a Dacron sterile cotton swab immediately after the first, was preserved in a transport medium containing sterile PBS solution. This second sample was designated for HPV DNA testing.

3.3.0 Cytological classification

The assessment of cytological findings was conducted based on the outcomes derived from the conventional Pap test, and the interpretations were aligned with the criteria outlined by the Bethesda system. Subsequent to the cytological diagnoses, patients were stratified into two principal groups, each adhering to specific inclusion criteria. The control group included patients who had cytological findings that were negative for intraepithelial lesions or malignancy (NILM).

The studied group encompassed patients with cervical lesions categorized into two subgroups: a group of women with cervical lesions associated with a high risk of progression to carcinoma (hrCLs), including: atypical squamous cells, findings where high-grade squamous intraepithelial lesions (ASC-H) cannot be excluded, high-grade squamous intraepithelial lesions (HSIL), squamous cell carcinoma (SCC), and a group of patients with cervical lesions associated with a low risk of progression to carcinoma (non-hrCL) including atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesions (LSIL).

Pap smears underwent meticulous analysis by skilled pathologists collaborating with family gynecologists to ensure precise and uniform interpretation of results in accordance with the criteria established by the Bethesda system.

3.4.0 Microbiological molecular analyses

3.4.1 Testing for the presence of DNA of 21 HPV types

DNA extraction was performed using a reagent kit designed for the extraction of genomic DNA, PureLink Genomic DNA, INVITROGEN, Life-Technology. The set of reagents is based on the principle of selective binding of DNA to silica membrane in the presence of chaotropic salts. Cells were digested with proteinase K at 55^{o}C using an optimized digestion buffer formulation that aids in protein denaturation and enhances proteinase K activity. Any residual RNA was removed by digestion with RNase A before

binding the samples to the silica membrane. The lysate was mixed with ethanol and a binding buffer that allows high binding of DNA to Spin Columns. DNA was bound to the silica membrane in the column and impurities were removed by thorough washing with two wash buffers. Genomic DNA was then eluted in elution buffer and stored at -20ºC until further use. The real-time multiplex polymerase chain reaction (PCR) was utilized for the detection of 21 HPV genotypes. This method enables the simultaneous detection of multiple targets (different HPV types) in a single reaction, ensuring specificity without non-specific cross-reactivity and maintaining a high level of sensitivity.

The implemented PCR method is based on the amplification of a specific DNA sequence. During the amplification cycles, DNA molecules undergo denaturation through heat. Target-specific primers, in the presence of nitrogenous bases and Tag-polymerase, bind to denatured DNA templates. Tag polymerase then extends the primers, facilitating the synthesis of complementary DNA strands and the amplification of the target DNA sequence. Real-time PCR technology is based on the measurement of fluorescence at each cycle of the reaction. Fluorescent dyes (FAM, ROX, and Cy 5) are utilized to detect individual specific HPV DNA sequences. The HEX color is employed for the detection of the internal control (IC), while the FAM color is utilized for the detection of the sample intake control (SIC). The use of several distinguishable colors enables the simultaneous detection of multiple PCR products in a single microtube. The fluorescence intensity is subsequently analyzed using a real-time PCR instrument, supported by appropriate software.

3.4.2 Measurement of HPV Viral Load

Quantification of HPV DNA, i.e. determination of the number of viral DNA copies per cell, is enabled by software that is compatible with HPV detection tests on the real-time PCR instrument, DTlite, LLC, Russia. This instrument enables two types of analysis: absolute (DNA copies/sample) and relative (DNA copies/10^5 epithelial cells) with automated interpretation. Absolute analysis involves a software calculation of the number of virus copies based on the threshold cycle (Cp) value after amplification. Relative analysis involves normalizing the amount of viral DNA to the number of human cells in the sample (SIC-sample intake control). This allows the sample variance to be taken into account.

In the analysis of the results of this study, relative quantification was used and the results were interpreted in logarithmic copies of HPV per 100,000 human cells (virus copies/human cells), where:

A viral load of less than 10^3 in relative quantification holds low clinical significance, indicating the potential for the human immune system to effectively clear the infection; considered a non-clinically significant, low viral load.

A viral load ranging from 10^3 to 10^6 in relative quantification is clinically valuable and denotes a risk of dysplasia; considered a moderate viral load.

A viral load exceeding 10^6 in relative quantification is clinically valuable and strongly suggests the presence of dysplasia; considered as a high viral load.

Results

Among the 300 women studied, 174 (58%) exhibited an infection caused by a single HPV type (ST-HPV), while 126 (42%) displayed an infection caused by multiple HPV types (MT-HPV).

Regarding age distribution, 54 patients (18%) were 24 years old or younger, 112 (37.3%) were between 25 and 34 years old, 89 (29.7%) were 35 to 44 years old, 35 (11.7%) were between 45 and 54 years old, and 10 (3.3%) were older than 55 years. The highest percentage of HPV positive samples was observed in women aged 25 to 34 years (37.3%), gradually decreasing with age. The analysis of patients with HPV infection, considering both

single and multiple types, revealed distinct age distributions. Notably, the percentage of patients with MT-HPV infection exhibited a bimodal distribution with peaks in the youngest group (up to 34 years) and in the oldest group (55 to 64 years). Within these age groups, infections caused by MT-HPV were significantly more prevalent compared to those caused by ST-HPV. Specifically, in patients aged up to 24 years, the percentage was 23.81% for MT-HPV *versus* 13.79% for ST-HPV. For individuals between 25 and 34 years old, the percentages were 38.10% for MT-HPV and 36.78% for ST-HPV. In the age group of 55-64 years, MT-HPV infection was 6.35%, while ST-HPV infection was 1.15%. Conversely, in the age groups of 35-44 years and 45-54 years, the prevalence of infections caused by ST-HPV was higher compared to those caused by MT-HPV, with percentages of 31.61% *versus* 26.98%, and 16.67% *versus* 4.76%, respectively (Table 1).

Table 1. Single and Multiple Human Papillomavirus Genotype Testing in Different Age Groups

Age Groups (years)	Total		ST-HPV* Infection		MT-HPV** Infection	
	N	(%)	N	(%)	N	(%)
≤ 24	54	18.00%	24	13.79%	30	23.81%
25-34	112	37.33%	64	36.78%	48	38.10%
35-44	89	29.67%	55	31.61%	34	26.98%
45-54	35	11.67%	29	16.67%	6	4.76%
55-64	10	3.33%	2	1.15%	8	6.35%
Total	300	100.00%	174	100.00%	126	100.00%

*ST: single type **MT: multiple types

To statistically assess the association between age and HPV infection types (ST-HPV and MT-HPV), a modified χ^2-test of independent signs was applied. The χ^2 value is 19.439, indicating a significant difference, and the p-value is 0.022, demonstrating a lower risk for error than the defined 0.05 level. Therefore, it can be concluded with statistical significance that ST-HPV infection and MT-HPV infection were dependent on the age structure of the patients.

In 97 women with normal cervical findings and 119 women with non-hrCL, ST-HPV infection was prevalent. Conversely, in 84 patients with hrCL co-infections with MT-HPV were predominant. Table 2 shows the summarized results of the cytological findings and the analysis between the different models of HPV infection (ST-HPV *vs*. MT-HPV infection). In women with ST-HPV infection, the percentage of patients from the control group, i.e., with a normal cervical finding was 68.04% (66 women) and was higher compared to the percentage of patients from the studied group, i.e., from patients who had non-hrCL with 58.86% (70 women) and from patients who had hrCL with 45.23% (38 women). In contrast, among women with MT-HPV infection, the proportion of patients with hrCL was 54.76% compared to 41.18% of patients with non-hrCL and 31.96% of patients with normal cervical findings. Statistical analyses using a modified χ^2-test of independent signs with an error risk of 0.05 and degrees of freedom (r), revealed that the cytological findings were independent of the number of determined HPV genotypes, ($p=0.124$) exceeding the defined risk for error ($1-\alpha$), i.e. 0.05.

Table 2. Single and Multiple type HPV Infection Status According to Cytological Groups

Cytology groups	1 HPV Genotypes		2 HPV Genotypes		3 HPV Genotypes		4+ HPV Genotypes		Total	
	N	%	N	%	N	%	N	%	N	%
Normal	66	68.04	14	14.43	10	10.31	7	7.22	97	100.0
Non-hrCL	70	58.82	23	19.33	14	11.76	12	10.09	119	100.0
hr-CL	38	45.24	21	25.00	12	14.29	13	15.47	84	100.0

*ST: single type **MT: multiple types

Table 3 provides an overview of the HPV types associated with infections caused by ST-HPV and MT-HPV. HPV16 emerged as the most prevalent type, identified in 71 patients, accounting for 13.08% of all HPV-positive cases. It exhibited the highest prevalence (22.29%) within the groups with ST-HPV infections. Other notable high-risk types included HPV31, HPV53, HPV51, and HPV44, with respective presence rates of 9.76%, 7.73%, 6.26%, and 6.08%. HPV18 was detected in only 21 patients, representing 3.87% of all HPV-positive cases.

Table 3. Representation of HPV Genotypes in Single and Multiple type HPV Infection

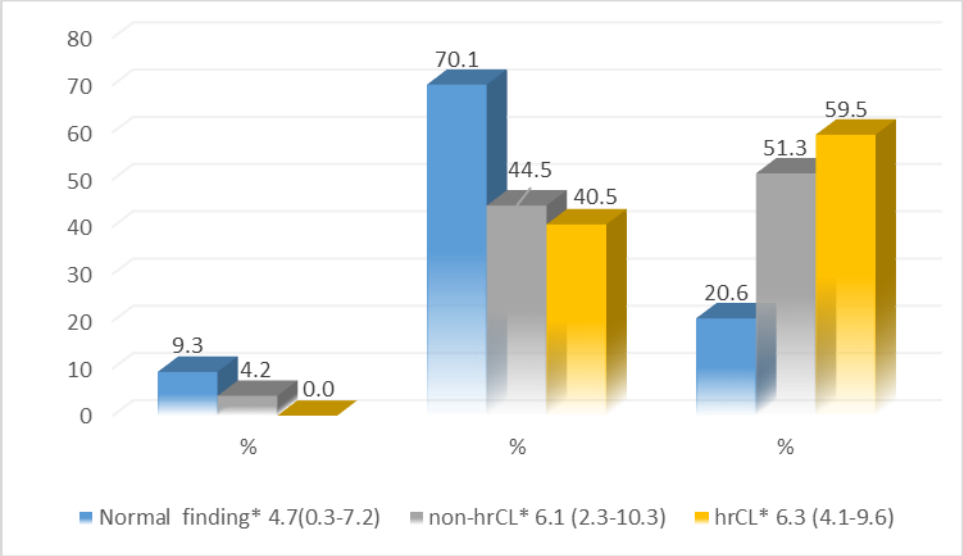
HPV Genotype	Total		ST-HPV* Infection		MT-HPV** Infection	
	N	%	N	%	N	%
6	22	4.05%	2	1.14%	20	5.41%
16	71	13.08%	39	22.29%	32	8.65%
18	21	3.87%	4	2.29%	17	4.59%
31	53	9.76%	21	12.00%	32	8.65%
33	15	2.76%	2	1.14%	13	3.51%
35	10	1.84%	5	2.29%	6	1.62%
39	23	4.24%	6	3.43%	17	4.59%
44	33	6.08%	9	6.29%	22	5.95%
45	20	3.68%	2	1.14%	18	4.86%
51	34	6.26%	12	6.86%	24	6.49%
52	32	5.89%	8	4.57%	24	6.49%
53	42	7.73%	9	5.14%	33	8.92%
56	22	4.05%	8	4.57%	14	3.78%
58	18	3.31%	4	2.29%	14	3.78%
59	27	4.97%	9	5.14%	18	4.86%
66	41	7.55%	16	9.71%	24	6.49%
68	27	4.97%	8	5.14%	18	4.86%
73	22	4.05%	6	4.00%	15	4.05%
82	10	1.84%	1	0.57%	9	2.43%
Total	543	100.00%	171	100.00%	370	100.00%

*ST: single type **MT: multiple type

To statistically assess the association between proportion of individual HPV types within ST-HPV infection and MT-HPV infection independent of the HPV type, modified χ^2-test was applied, a test of independent signs, with an error risk of 0.05 and degrees of freedom (r) calculated as (m-1) (n-1) = (19-1) (2-1) = 18; the theoretical (critical) value of the test was 28.869. As (χ_pr^2=40.806) exceeded (χ (0.05;18)^2=28.869), it can be concluded that the proportion of individual HPV types within ST-HPV infection and MT-HPV infection did depend on the HPV type. This conclusion is further supported by the fact that the defined

risk for error (1-α), i.e., 0.05, was greater than the realized level of the risk for error, represented by p=0.001.

Regarding HPV viral load, 14 patients (4.7%) had a low viral load (transformed log10 <3.0 copies/sample), 155(51.7%) had an intermediate viral load (transformed log 10-3.1-6.0 copies/sample), and 131(43.7%) had a high viral load (transformed log10 >6.1 copies/ sample). Differences in HPV DNA concentration were observed between the control and study groups, with the severity of cervical lesions positively correlating with viral load. The percentage of patients with detected high concentration of HPV DNA viral load in the cells was higher in the study group compared to patients of the control group, i.e., the percentage of patients with hrCL and non-hrCL was 59.5% and 51.3% respectively, and was higher in relationship to the percentage of patients with a normal cervical finding, which was 20.6%. Statistical analysis of the data suggests a statistically significant association between the severity of cervical lesions and HPV DNA viral load, since the calculated chi-square value (143.76) exceeded the critical value (5.99). This conclusion is further supported by the fact that the defined risk for error (1-α), i.e., 0.05, was greater than the realized level of the risk for error, represented by p=0.0001 (Figure 1).



*Log 10 - transformed viral load

Fig. 1. Percentage of women with cytologic abnormalities by quantitative HPV DNA viral load

Table 4 illustrates the correlation between type-specific viral load in relation to cervical lesions. The viral loads of individual HPV types (HPV-16, -31, -51, -52, and -58), expressed as logarithmic HPV DNA copies per 100,000 human cells, exhibited a clear linear increase as cervical cytologic status progressed from normal to lesions with a high risk for malignancy. For instance, viral loads for HPV-16 ranged from 4.8 to 5.6, HPV-31 showed values of 4.9 to 5.9, HPV-51 displayed values of 4.8 to 6.9, and values for HPV-52 varied from 4.8 to 6.8. Viral loads for HPV-58 were 4.0, 4.5, and 4.9. Interestingly, viral loads for HPV-18, HPV-45, HPV-56, and HPV-59 did not increase but rather decreased with worsening cervical lesions.

Moreover, the individual viral loads of HPV-16, -31, -35, -51, -52, -53, -58, -59, and -73 were significantly higher in women with high-risk cervical lesions compared to those with normal cytology. Compared to patients with non-hrCL, those with hrCL showed significantly

higher viral loads for HPV-16 (5.4 *vs*. 5.6), HPV-31 (5.2 *vs.* 5.9), HPV-35 (4.9 *vs.* 5.6), HPV-51 (5.9 *vs*. 6.9), HPV-52 (6.1 *vs.* 6.8), and HPV-58 (4.5 *vs*. 4.9), as listed in Table 4.

Table 4. Correlation analyses of the relationship between type-specific HPV DNA viral load and severity of intraepithelial lesions

HPV Genotype	Normal group		Non-hrCL**		hrCL***	
	No. of women	HPV DNA viral load*	No. of women	HPV DNA viral load	No. of women	HPV DNA viral load
6	5	5.1	9	4.6	5	4.6
16	22	4.8	35	5.4	14	5.6
18	8	4.4	8	3.8	5	2.6
31	16	4.9	15	5.2	23	5.9
33	3	4.8	9	6.7	3	4.9
35	4	4.5	5	4.9	1	5.6
39	7	3.4	11	4.9	0	0.0
44	14	4.8	12	4.8	8	4.1
45	3	4.6	11	4.4	4	4.7
51	8	4.8	16	5.9	12	6.9
52	5	5.8	16	6.1	11	6.8
53	14	4.5	17	5.5	12	5.2
56	8	5.0	5	5.1	9	5.2
58	5	4.0	8	4.5	5	4.9
59	11	3.9	10	6.6	6	5.1
66	14	5.5	14	5.3	13	5.8
68	13	4.6	11	4.2	3	4.1
73	6	1.9	7	5.4	9	5.4
82	3	5.5	5	5.8	2	5.2

*Log 10 - transformed viral load; ** Non-hrCL - non high risk cervical lesions; *** hrCL - high risk cervical lesions

To statistically assess if different HPV genotypes and various cytological findings from the Pap test affected the concentration of HPV DNA, the parametric ANOVA test with two factors and multiple modalities was employed.

The theoretical value of the Snedecor variable F(R) for 18 and 36 degrees of freedom, with a significance threshold of 0.95, pertaining to different HPV genotypes, was 1.898622. The calculated value of the Snedecor variable F(R) was 2.215491, which exceeded the theoretical value. Therefore, it can be concluded that different HPV genotypes did impact the concentration of HPV DNA. This conclusion is supported by the fact that the defined risk for error (1-α), i.e., 0.05, was greater than the realized level of the risk for error (p=0.020756).

Discussion

This study investigated the correlation between ST-HPV and MT-HPV infections, age, and HPV DNA viral load in conjunction with cytological findings. In this study, 300 women were tested for 21 HPV genotypes. The results showed that patients with HPV infection caused by ST-HPV and MT-HPV showed a different age distribution. It is important to note that the percentage of patients with viral infection caused by MT-HPV showed a bimodal pattern with two distinct peaks. The first peak was observed in the youngest group of patients, which included women aged up to 34 years, while the second peak was observed in the oldest group, which included women aged 55 to 64 years. In these mentioned age groups, infections caused by MT-HPV were represented in a significantly higher percentage compared to infections caused by ST-HPV: in patients aged up to 24 years the percentage was 23.81% *versus* 13.79% respectively, in the age group of 25 -34 years old it was 38.10% *versus* 36.78%, and in patients from the age group of 55-64 years, the percentage was 6.35% *versus* 1.15%. These findings are consistent with other studies, such as that of Resende *et al*[22]. This

study showed a bimodal prevalence curve for MT-HPV infections with prevalence peaks in the very young (<29 years) and in women aged 50-59 years. But there are studies, such as that of Salazar *et al.*[19], where the percentage of MT-HPV infections was found to be the highest at the age of 25-34 years, and then gradually decreases.

Several published studies have shown that the risk of developing hrCL decreases with increasing number of genotypes with which the cervix is co-infected, especially when infections include 3 or more HR- HPV genotypes, explaining that the weak and ineffective immunity generated by each genotype of HPV may collectively provide an overall stronger immunity against HPV infection through antibody cross-reactions[19]. However, other authors found a significant association between MT-HPV infections and disease severity and concluded that infection with multiple HPV types was a significant risk factor for high-grade CIN[20]. These claims are consistent with the results of this study, where, despite the limited sample size, MT-HPV infections exhibited a significantly higher prevalence than ST-HPV infections in patients with hrCL. Specifically, the percentage of MT-HPV infections was the highest (54.76%) in patients with hrCL compared to patients with non-hrCL (41.18%) and those with normal cervical findings (31.96%).

In the realm of research on the correlation between viral loads of HR-HPV and cervical lesions, a substantial body of evidence has been amassed, with numerous studies reporting that HPV DNA viral load varies directly with the degree of cervical lesions. Long *et al.*[23] reported that a higher viral load of HPV 16 in cells was associated with a risk of CIN 3, but cervical cytology varied from the time when the amount of HPV DNA was measured. Furthermore, Li *et al.*[21], in a study examining the presence of individual HPV genotypes and their viral load in correlation with cervical lesions, reported that there were significant differences between HPV types in the distribution of HPV infections caused by ST or MT, and there was a positive correlation between viral load and pathological grade of cervical lesions when infections were caused by HPV 16, 18, 31, 33, 51, 52, 53 and 58. In a similar study, Dong[24] and colleagues proved that there was a positive correlation between HPV -16, -31, -33, -52 and -58 DNA viral load and cervical lesion severity, while DNA viral load of HPV-18, -45, -56, -59 was not positively correlated. The latter claims are consistent with the results obtained in this study, where viral loads of individual HPV types, such as HPV-16, -31, -51, -52, and -58, were found to clearly show a linear increase as changes in the cervical cytological status from normal to cervical lesions with a high degree of risk of malignancy. Surprisingly, viral loads for HPV-18, HPV-45, HPV-56, and HPV-59 decreased with progression of cervical lesion findings. Contrary to the given claims, there are studies, such as that of Del Rio Ospina[25] and colleagues, who reported that cervical lesions were more common in patients with a low concentration of HPV -16 and -31 DNA.

Conclusion

This study underscores the critical significance of early diagnosis in preventing cervical cancer. By investigating the interplay between patient age, infection with various HPV genotypes, the association between HPV viral load and cervical lesion severity, our findings offer essential insights. These revelations hold immense value in guiding appropriate triage measures, particularly in minimizing unnecessary examinations for women with normal cytology experiencing a transient infection with HR-HPV types. Implementing this approach could revolutionize the risk stratification within cervical cancer screening programs, furnishing crucial information to enhance the precision and effectiveness of targeted screening strategies.

Conflict of interest statement. None declared.

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