ORIGINAL RESEARCH ARTICLE

Prevalence of human papillomavirus infection among women at a gynaecological clinic in Tlemcen, Algeria

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Abstract

High-risk human papillomavirus (HR-HPV) is recognized as the etiologic agent responsible for cervical cancer, ranking as the second most prevalent cancer among women in Algeria with an incidence rate of 10.4 per 100,000. The primary aim of this study was to conduct a preliminary prospective investigation into the detection of HR-HPV infections in Tlemcen, Algeria, where screening is exclusively based on cytology. A total of 130 cervical swabs were analysed in this study. HPV detection was performed utilizing the Cobas® 4800 test, incorporating polymerase chain reaction (PCR) for individual genotyping of HPV-16 and HPV-18, as well as pooled detection of 12 other commonly occurring HPVs. The findings revealed that out of the 130 samples, 28 tested positive for HR-HPV, resulting in a prevalence rate of 21.5%. Among these cases, five infections demonstrated the coexistence of HPV16 with other HR-HPV genotypes. The prevalence of HPV16 infections was determined to be 28.6% (8/28), whereas 68% of infections (19/28) were attributed to other HR-HPV genotypes. These observations indicate that HPV16 was not the prevailing genotype. Consequently, these results underscore the necessity for a larger-scale study with an expanded sample size encompassing cytology and HPV testing. Such an investigation would be invaluable in facilitating the development of a national prevention program to effectively control cervical cancer. *(Afr J Reprod Health 2023; 27 [7]: 56-63).*

Keywords: Cervical cancer, screening, HR-HPV, PCR

Résumé

Le papillomavirus humain à haut risque (HR-HPV) est reconnu comme l'agent étiologique responsable du cancer du col de l'utérus, se classant comme le deuxième cancer le plus répandu chez les femmes en Algérie avec un taux d'incidence de 10,4 pour 100 000. L'objectif principal de cette étude était de mener une enquête prospective préliminaire sur la détection des infections à HR-HPV à Tlemcen, en Algérie, où le dépistage est exclusivement basé sur la cytologie. Au total, 130 écouvillons cervicaux ont été analysés dans cette étude. La détection du VPH a été réalisée en utilisant le test COBAS® 4800, incorporant la réaction en chaîne par polymérase (PCR) pour le génotypage individuel du HPV-16 et du HPV-18, ainsi que la détection regroupée de 12 autres HPV courants. Les résultats ont révélé que sur les 130 échantillons, 28 testés positifs pour HR-HPV, résultant en un taux de prévalence de 21,5%. Parmi ces cas, cinq infections ont démontré la coexistence de HPV16 avec d'autres génotypes HR-HPV. La prévalence des infections à HPV16 a été déterminée à 28,6% (8/28), tandis que 68% des infections (19/28) ont été attribuées à d'autres génotypes HR-HPV. Ces observations indiquent que HPV16 n'était pas le génotype dominant. Par conséquent, ces résultats soulignent la nécessité d'une étude à plus grande échelle avec une taille d'échantillon élargie englobant la cytologie et les tests de VPH. Une telle enquête serait inestimable pour faciliter le développement d'un programme de prévention national pour contrôler efficacement le cancer du col de l'utérus. *(Afr J Reprod Health 2023; 27 [7]: 56-63).*

Mots-clés: Cancer du col de l'utérus, dépistage, HR-HPV, PCR

Introduction

Cervical neoplasia is one of the cancers whose incidence is steadily decreasing in developed countries, while it is increasing in low-resource countries[1]. Cervical cancer is the second most common female cancer in the world in terms of incidence and the first in terms of mortality[2,3]. This mortality is particularly high in Africa where less than 50% of women with cervical cancer survive beyond five years[1,4]. In Algeria, its incidence is 10.4 per 100,000 women according to Globocan[5].

Estimates indicate that each year, 1726 women are diagnosed with cervical cancer and 1391 die from the disease in Algeria[6]. A total of 196 cases of cervical cancer were recorded in the Wilaya region of Tlemcen between 2006 and 2010 with an incidence of 8.3 per 100,000 women according to the cancer registry of the Wilaya. The majority of cases was invasive with high cost of treatment[7]. Cervical cancer therefore represents one of the most serious attacks on the health of women in countries with limited resources[2].

Preceded by numerous precancerous lesions, which are most often asymptomatic, cervical cancer is a pathology particularly accessible to screening[8,9]. The screening policy has been based since the 1950s mainly on the detection of cytological (smear) or histological (biopsy) abnormalities, or even from colposcopy. But these morphological approaches based on subjective assessment are a source of false negatives and false positives, hence a lack of reproducibility. Cervical cancer screening in some developed countries currently benefits from the HPV test whose excellent negative predictive value improves screening sensitivity[10,11,12]. Used in addition to the smear, the HPV test would be more sensitive than cytology alone and would make it possible to limit the frequency of screening tests. Infections detected by highly sensitive molecular methods have a prevalence three to ten times higher than that of intraepithelial lesions detected by cytology[11,13].

HPV-HR identified in 97 to 99% of cancers of the cervix, are the main risk factors for the occurrence of cancer of the cervix[14,15], the most frequent of which are HPV 16, 18, 31, 33, 45[9]. According to a study carried out in Algiers, the prevalence of HPV in the general population is 6.3%, including 4.0% high-risk types[16]. No study concerning the prevalence of HPV has been carried out at the level of the Wilaya of Tlemcen, hence the interest of this preliminary study which consists of a search for HPV-HR on cervical samples at the level of the Wilaya of Tlemcen where screening is based exclusively on cytology.

Methods

A total of 130 cervical samples were collected from female patients aged between 23 and 72 years.

Among them, 98 samples were obtained during gynaecological consultations at various health centres and private practices in the Wilaya of Tlemcen. Additionally, 32 samples were collected from seropositive patients attending the infectious disease department of the University Hospital of Tlemcen. In parallel to obtaining cervical-uterine smears (FCU) on a slide, samples intended for HPV research were obtained using a cyto-brush and introduced into the PreservCyt® solution (Cytyc Corporation, Boxborough, USA). An information sheet was created for each patient, which included personal information, the visual appearance of the cervix as determined by the sampler, and the subsequent cytological diagnosis reported by the cytopathologist.

Participants

The study participants were randomly selected from a pool of patients who were already undergoing cytology tests. To ensure random selection, a systematic and unbiased method was employed. Inclusion criteria for participation in the research on human papillomavirus (HPV) included obtaining patient consent, which was obtained through the signing of a consent approval form.

Cytology

The cytology examinations for women in this study followed a standardized protocol recommended by the institution. Trained and specialized physicians performed cervical sample collection using a cyto-brush or spatula, obtaining cellular material from the cervix. The collected samples were then transferred onto glass slides and fixed with an appropriate fixative, such as alcohol-based or spray fixative solutions. Following fixation, the slides were processed using the Papanicolaou staining method or an equivalent staining technique to visualize cellular morphology. The stained slides were then examined under a microscope by skilled cytotechnologists or cytopathologists for the detection and evaluation of cellular abnormalities, including any indicators of human papillomavirus infection or cervical neoplasia. The cytology results were interpreted and classified according to established diagnostic criteria, such as the Bethesda system or an equivalent classification system,

providing valuable information for the assessment of cervical health and potential abnormalities.

HPV assay

HPV detection was performed using the Cobas HPV 4800® test (Roche® molecular systems, Inc.). It is a qualitative in vitro test that allows the amplification of target DNA by PCR and nucleic acid hybridization. This test specifically identifies types 16 and 18, and simultaneously detects a pool of the 12 most common HR-HPVs (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). The Cobas® HPV Test is based on two major processes: (1) Automated sample preparation to simultaneously extract HPV and cellular DNA. (2) PCR amplification of target DNA sequences using both HPV and β-globin complementary consensus specific primer pairs as a control, as well as real-time detection of labelled probes by fluorescence specific to HPV and β-globin[17,18,19].

Data analysis

The collected data were downloaded as an Excel file and subsequently subjected to a thorough cleaning and analysis process. The IBM SPSS Statistics software, version 22, was utilized for data management and statistical analysis. Prior to analysis, the dataset underwent careful cleaning, including data validation, removal of outliers, and handling of missing values. Descriptive statistics, such as means, standard deviations, frequencies, and percentages, were calculated to summarize the characteristics of the study population and variables of interest. Inferential statistics, including parametric or non-parametric tests, were performed to explore relationships, differences, or associations among variables. The statistical significance level was set at p < 0.050, indicating that findings with p-values below this threshold were considered statistically significant.

Ethical consideration

Research ethical issues including informed consent, anonymity, and confidentiality, were addressed carefully during the study process. The research ethical clearance approval letter was obtained from the Scientific Research Ethics Committee of University of Tlemcen, Algeria.

Results

The socio-demographic characteristics of the study participants are presented in Table 1. The average age of the women was 40.9 years, with over 50% of them aged 40 years or older. The majority of women (65.4%) have their first sexual intercourse at or before the age of 20. The number of pregnancies ranged from 0 to 7, with an average of 3.6 pregnancies per woman. About 53.8% of women had more than three births, and a history of miscarriage was recorded in 38.5% of cases. Oral contraception was the most commonly used method (57.7%), and approximately 70% of the participants were undergoing their first Pap smear, often at a later age. The main reason for gynaecological consultations was functional signs, primarily bleeding. Approximately 18% of the patients were HIV positive, representing individuals from the infectious disease department (CHU Tlemcen) (Table 1).

The Cobas® HPV 4800 test identified 28 positive HPV samples, resulting in an overall infection rate of 21.5%. Among these, 5 infections were associated with HPV16 and other high-risk HPV (HR-HPV) types. The rate of infection by HPV16 alone or in combination was 28.6% (8/28), while HPV16 and/or 18 were detected in 32.2% (9/28) of cases. The remaining 68% of infections were attributed to other HR-HPV genotypes, with 19 out of 28 samples testing positive (Table 2).

The frequency of HPV infection varied across different age groups. Among patients aged 30 to 39, the infection rate was 31.9% (15/47), which decreased to 10% (4/40) in the 40-49 age group. However, a peak rate of 35.7% (5/14) was observed among women aged 50-59. Furthermore, patients with more than three pregnancies had an infection rate of 25.7% (18/70), and those who married at or before the age of 20 had a rate of 25.9% (22/85). Among women using oral contraceptives, the infection rate was 24% (18/75), while those undergoing their first Pap smear had an infection rate of 24.4% (22/90). Notably, the highest infection rate was observed in HIV-positive

Table 1: Characteristics of patients participating in the cytology analysis and frequency of HPV infection

CARACTERISTICS	EFFECTIVE	%	PCR+	%
Age	**Mean Age: 40,9 (23 - 72)**			
<30	17	13,1	4	23,5
30-39	47	36,2	15	31,9
40-49	40	30,8	4	10
50-59	14	10,8	5	35,7
≥60	12	9,2	0	0
Age of first sexual intercourse	**Mean Age: 20,3 (14 - 40)**			
≤ 20	85	65,4	22	25,9
> 20	45	34,6	6	13,3
Gestation	**Mean of Gestation: 3,6 (0 - 7)**			
≤ 3	60	46,2	10	16,7
> 3	70	53,8	18	25,7
Miscarriage				
Yes	50	38,5	12	24
No	80	61,5	16	20
Contraception				
Oral	75	57,7	18	24,0
Neither	28	21,5	6	21,4
Other	27	20,8	4	14,8
Pap smear frequency				
1st smear	90	69,2	22	24,4
Control smear	40	30,8	6	15
Appearance of the collar				
Bleeding on contact	50	38,5	14	28
Normal	31	23,8	8	25,8
Other	49	37,7	6	12,2
Seropositivity				
HIV+	32	17,8	18	56,3
HIV -	98	82,2	10	10,2

Table 2: Frequency of HPV infection

HPV	EFFECTIVE	%
HPV 16	3	10,7
HPV 18	1	3,6
HPV16 + HPV18	1	3,6
HPV16 + Other HPV-HR	3	10,7
HPV16 + HPV18 + Other HPV-HR	1	3,6
Other HPV-HR	19	67,9
Total	**28**	**100**

patients, with 56.3% (18/32) testing positive, compared to 10.2% (10/98) among patients in gynaecological consultation.

The relationship between HPV infection and cytological profiles is presented in Table 3. Among the normal smears, 16.7% (5/30) were positive for HPV, including 4 cases in HIV-positive patients. Among those with an inflammatory smear, 29.4% (10/34) were HPV positive. The infection rate was 61.5% (8/13) in patients with high-grade lesions, whereas only 5.4% of patients with low-grade lesions tested positive for HPV, with 2 out of 37 cases being HPV positive. Out of the 3 patients with squamous cell carcinoma, 2 (66.7%) were HPV positive, including one patient without treatment and one with post-therapeutic recurrence. It is worth noting that HPV was not detected in any of the cellular atypia cases.

Overall, the results demonstrate a significant prevalence of HR-HPV infection among the study population. The correlation between HPV infection and cytological profiles suggests a higher risk of high-grade lesions and squamous cell carcinoma among HPV-positive individuals. The identified risk factors, such as age, number of pregnancies, early age at first sexual intercourse, oral contraceptive use, and HIV status, provide valuable insights into the determinants of HPV infection. Further analysis, including bivariate and multivariate analysis, will be conducted to assess the associations between these risk factors and positive HPV and abnormal cytology results,

Table 3: Relationship between cytologic profile and the frequency of HPV infection

CYTOLOGICAL PROFILE	EFFECTIVE	%	PCR+	%
Normal	30	23,1	5	16,7
Inflammatory	34	26,2	10	29,4
Atrophy	6	4,6	1	16,7
Cellular atypia	7	5,4	0	0
Low-grade lesion	37	28,5	2	5,4
High grade lesion	13	10	8	61,5
Squamous cell carcinoma	3	2,3	2	66,7
Total	**130**	**100**	**28**	**21,5**

facilitating a comprehensive understanding of the prevalence, correlation, and predictive factors of HR-HPV infection and abnormal cytology results.

Discussion

In this preliminary prospective study focusing on the detection of high-risk human papillomavirus (HR-HPV) with partial genotyping (HPV16 and 18), an infection frequency of 21.5% was identified. This rate was notably high, particularly considering the presence of HIV-positive cases. Among patients in gynaecological consultation excluding HIV-positive individuals, the infection frequency was 10.2%, higher than the prevalence recorded in Algiers by Hammouda et al. (2011), which was 6.3%. These findings indicate a higher prevalence of HR-HPV in the study population compared to other regions, resembling prevalence rates observed in Europe rather than sub-Saharan Africa[16]. Comparing our results with studies conducted in other countries, the prevalence of HR-HPV infections was significantly lower than that found in Morocco, where the prevalence reached 45.6%[20]. According to Castellsagué *et al.*, the estimated prevalence of HR-HPV infections in North Africa is around 21.5%[6]. Notably, the prevalence of HPV infection varies significantly across countries, ranging from 1.4% in Spain to 25.6% in Nigeria, according to a study conducted by the International Agency against Cancer[21]. Furthermore, there are variations in HPV prevalence within the same country, as observed by Bennani *et al.* in Morocco, where Fez recorded a prevalence of 47% compared to other cities, which had an 18% prevalence. These variations indicate that the risk of HR-HPV infection is multifactorial and dependent on geographic and population-specific factors[20].

Our study identified five infections associating HPV16 with other HR-HPV genotypes, representing 17.9% of the cases. Studies have suggested that HPV16 infection is associated with an increased risk of subsequent acquisition of other HPV types[22,23,24], and certain HPV types may interact or act synergistically to induce lesion development or progression[25,26]. In this study, the detection of multiple HR-HPV types was more common in HIV-positive patients compared to immunocompetent women, which is consistent with previous findings.

While the two HR-HPVs covered by the currently available vaccination, HPV16 and 18, were detected in 32.2% of the cases (either alone or in combination), nearly 68% of the infections were caused by other HR-HPV genotypes. This indicates that HPV16 was not the predominant genotype, in contrast to most previous studies where HPV16 prevalence was predominant, followed by HPV18, HPV31, and HPV45[30]. The distribution of HPV types varies geographically, and limited data exist on the epidemiological and virological characteristics of cervical cancer in North Africa, particularly in Algeria. Previous studies conducted in Algiers have reported different predominant HPV types, with HPV16 being the most common type in one study[31] and HPV31 in another[16]. The heterogeneity in HPV type distribution among different populations should be considered when developing tests and predicting the impact of vaccines on infection incidence[21].

Age-specific HPV prevalence data are crucial for understanding trends in HPV prevalence across different regions. Generally, HPV prevalence decreases with age after reaching a peak in young women ($\leq$ 25 years)[32]. However, certain geographic regions may exhibit a second peak

among elderly women[32,33,34]. Our study also observed a second peak among middle-aged women, which indicates variations in HPV prevalence based on geographic location and sexual behaviors[33].

In addition to HR-HPV infection, various cofactors can contribute to the acquisition, persistence, and progression of cervical cancer[35]. These cofactors include early age at onset of sexual intercourse, lifetime number of sexual partners, the high number of pregnancies (more than 3), smoking, long-term use of oral contraceptives, and history of sexually transmitted infections (such as Chlamydia, HSV, HIV, etc.)[36]. In our study, the rate of HPV infection was higher in patients with more than three pregnancies (25.7%), those who married at age 20 or less (25.9%), those using oral contraceptives (24%), and immunocompromised patients (56.3%). HIV-positive women are at a higher risk of HPV infection, persistence, and progression to cervical cancer[37]. Therefore, systematic screening for cervical cancer should be implemented for all HIV-infected women.

Regarding the diagnostic accuracy of the cytological examination, our study found that 61.5% of high-grade lesions were HPV-positive. However, for patients who tested negative for HPV, the abnormalities detected by cytological examination may be unrelated to the 14 HR-HPV types screened in our study or could be attributed to interpretation errors. It is well-recognized that diagnoses based on visual interpretation have limitations, including reproducibility and subjectivity issues[10,38]. False positives can lead to patient distress, unnecessary additional examinations, and sometimes overtreatment[38]. In contrast, the high negative predictive value of the HPV test[11] indicates that patients with a negative result have a shallow risk of developing cervical cancer in the following years (3 to 6 years)[39], as the ability of the HPV test to exclude underlying lesions is greater than 99%[10].

In our study, two out of three patients with squamous cell carcinoma were HPV positive, including one without treatment and another with post-therapeutic recurrence. The sensitivity of HPV testing following surgery appears to be higher than that of Pap smear and colposcopy. Therefore, HPV testing should be integrated into postoperative monitoring for cervical lesions[30].

Conclusion

At the end of this preliminary prospective study, an HPV infection rate of 21.5% was recorded, with 10.2% in patients in gynaecological consultation, where HPV16 was not the predominant genotype. with 67.9% of infections were due to other HR-HPVs. The risk factors that seem to be linked to HPV infection were, in addition to seropositivity, the absence of early and regular screening, where the majority of patients has their smears taken for the first time at an advanced age whose main reason of gynaecological consultations were functional signs, mainly haemorrhage on contact, in addition to the excessive use of oral contraception and the early age of marriage. The introduction of the HPV test remains very important in screening for cervical cancer, where HPV + patients must be rigorously monitored for proper management. For HPV - patients; the risk of developing cancer in the next few years is almost nil. In addition, the HPV vaccine is a great hope in terms of public health, because it can effectively prevent the second cancer in women. If vaccination is a hope, it is on the condition that it comes in addition to screening and cannot replace it.

Competing interests

The authors declare no conflict of interest.

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