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High-Risk Human Papillomavirus Genotypes and Their Association with Cervical Cytological Changes among Women in Benghazi: A Molecular Cross-Sectional Study

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الأنماط الجينية لفيروس الورم الحليمي البشري عالي الخطورة وارتباطها بالتغيرات الخلوية العنقية بين النساء في بنغازي: دراسة جزيئية مقطعية

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Abstract

Cervical cancer is strongly associated with persistent infection by high-risk human papillomavirus (HR-HPV). This molecular cross-sectional study aimed to determine the prevalence and distribution of HR-HPV genotypes and their association with cervical cytological changes among women in Benghazi, Libya. Cervical specimens were collected from 300 women aged 21–65 years. Cytological findings were classified according to the Bethesda System, while HPV DNA was detected and genotyped using real-time polymerase chain reaction. HR-HPV was identified in 51 women, giving an overall prevalence of 17.0%. HPV-16 was the predominant genotype, followed by HPV-18, HPV-31, and HPV-52. Abnormal cytology was observed in 17.0% of participants, with LSIL and ASC-US being the most frequent abnormalities. HR-HPV positivity increased with cytological severity and reached 75.0% among women with HSIL. The findings demonstrate a significant association between oncogenic HPV infection and cervical abnormalities, supporting the integration of molecular HPV testing with cervical cytology in effective screening programmes.

Keywords: Human papillomavirus; High-risk HPV; HPV genotypes; Cervical cytology; Cervical lesions; Benghazi.

الملخص

يرتبط سرطان عنق الرحم ارتباطًا وثيقًا باستمرار العدوى بالأنماط الجينية عالية الخطورة لفيروس الورم الحليمي البشري هدفت هذه الدراسة الجزيئية المقطعية إلى تحديد معدل انتشار الأنماط عالية الخطورة وتوزيعها، وبيان علاقتها بالتغيرات الخلوية في عنق الرحم لدى النساء بمدينة بنغازي، جُمعت مسحات عنق الرحم من 300 امرأة تراوحت أعمارهن بين 21 و65 سنة. صُنفت النتائج الخلوية وفق نظام بيثيسدا، بينما كُشف عن الحمض النووي للفيروس وحددت أنماطه باستخدام تفاعل البوليميراز المتسلسل في الوقت الحقيقي ظهرت العدوى عالية الخطورة لدى 51 امرأة، بمعدل انتشار بلغ 17.0% كان النمط HPV-16 الأكثر شيوعًا، تلاه HPV-18 ثم HPV-31 وHPV-52. وظهرت تغيرات خلوية غير طبيعية لدى 17.0% من المشاركات، وكانت الآفات الحرشفية منخفضة الدرجة والخلايا الحرشفية غير النمطية الأكثر تكرارًا ارتفعت إيجابية الفيروس مع شدة التغيرات الخلوية، وبلغت 75.0% بين حالات الآفات عالية الدرجة تؤكد النتائج وجود علاقة مهمة بين العدوى المسرطنة والتغيرات الخلوية، وتدعم دمج الفحص الجزيئي مع الفحص الخلوي ضمن برامج الكشف المبكر.

الكلمات المفتاحية: فيروس الورم الحليمي البشري؛ الأنماط عالية الخطورة؛ الأنماط الجينية؛ فحص عنق الرحم الخلوي؛ آفات عنق الرحم؛ بنغازي.

Introduction

Cervical cancer remains a major public health concern and represents one of the most prevalent malignancies among women, particularly in low- and middle-income countries where access to organized screening and early diagnostic services is limited. Persistent infection with oncogenic human papillomavirus (HPV) is recognized as the principal cause of cervical intraepithelial neoplasia and invasive cervical cancer. Although most HPV infections are transient and spontaneously eliminated by the immune system, persistent infection with high-risk HPV genotypes can initiate progressive epithelial alterations that may eventually develop into malignant lesions [1].

Human papillomavirus is a small, non-enveloped virus with a circular double-stranded DNA genome. More than 200 HPV genotypes have been identified, approximately 40 of which are capable of infecting the anogenital epithelium. Genital HPV genotypes are classified according to their oncogenic potential into low-risk and high-risk groups. HPV-16 and HPV-18 are considered the most clinically important oncogenic genotypes, while HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, and HPV-59 also contribute to the development of high-grade cervical lesions and invasive cervical cancer [2].

The development of cervical cancer following high-risk HPV infection is generally a prolonged, multistep process. Persistent infection promotes the expression of the viral E6 and E7 oncogenes, which interfere with essential cellular regulatory mechanisms, particularly those involving the p53 and retinoblastoma proteins. Disruption of these pathways facilitates uncontrolled cellular proliferation, resistance to apoptosis, genomic instability, and the progressive accumulation of epithelial abnormalities. Therefore, detecting high-risk HPV DNA is valuable for identifying women at an increased risk of cervical precancerous and cancerous changes [3].

Cervical cytology has traditionally been used to detect epithelial abnormalities before their progression to invasive disease. Cytological findings are commonly classified according to the Bethesda System as negative for intraepithelial lesion or malignancy, atypical squamous cells of undetermined significance, low-grade squamous intraepithelial lesion, high-grade squamous intraepithelial lesion, or glandular abnormalities. However, the diagnostic performance of

cytology can be influenced by specimen adequacy, slide preparation, staining quality, and interpretive experience [4].

Molecular HPV testing provides an opportunity to identify oncogenic viral infections before evident morphological abnormalities appear in cervical cells. Combining HPV DNA detection with cytological examination may improve the identification and clinical classification of women who require additional diagnostic evaluation. The World Health Organization recommends HPV DNA detection as an effective primary cervical screening method because of its greater sensitivity for detecting women at risk of developing high-grade cervical lesions [5].

Polymerase chain reaction-based methods have substantially improved the molecular detection and characterization of HPV. These techniques offer high analytical sensitivity and allow the identification of individual HPV genotypes and the detection of single or multiple infections. Genotype-specific information is epidemiologically important because HPV distribution varies across geographical regions and populations. Such information may support the development of locally appropriate screening strategies and help estimate the potential coverage of prophylactic HPV vaccines [6].

Studies conducted among African women have demonstrated considerable variation in HPV prevalence and genotype distribution across different cytological categories. The prevalence of HPV generally increases with the severity of cervical abnormalities. HPV-16 is frequently reported as the predominant oncogenic genotype; nevertheless, HPV-18, HPV-31, HPV-35, HPV-45, HPV-52, and HPV-58 also contribute substantially to cervical infection and neoplastic changes in African populations [7].

Statement of the Problem

High-risk human papillomavirus infection is the principal factor associated with cervical precancerous and cancerous lesions. However, information regarding the prevalence and distribution of high-risk HPV genotypes among women in Benghazi remains limited. The relationship between these genotypes and cervical cytological changes has also not been adequately investigated. This lack of local molecular and cytological data limits the identification of the most prevalent oncogenic genotypes and the development of appropriate cervical cancer screening and prevention strategies. Therefore, this study investigates high-risk HPV genotypes and their association with cervical cytological changes among women in Benghazi.

ignificance of the Study

This study provides local molecular evidence on the prevalence and distribution of high-risk HPV genotypes among women in Benghazi. It also clarifies the association between HPV infection and different cervical cytological findings. The results may contribute to identifying women at increased risk of cervical lesions, improving early detection practices, and supporting the integration of molecular HPV testing with cervical cytology. Moreover, the findings may provide baseline data for future research and assist health authorities in developing locally appropriate screening, vaccination, and cervical cancer prevention programmes.

Aim of the Study

This study aims to determine the prevalence and distribution of high-risk HPV genotypes and assess their association with cervical cytological changes among women in Benghazi.

Specific Objectives

1. To determine the prevalence of high-risk HPV infection among women in Benghazi.
2. To identify the distribution of high-risk HPV genotypes among the study participants.
3. To describe the cervical cytological findings among the examined women.

4. To examine the association between high-risk HPV infection and cervical cytological changes.
5. To determine the relationship between individual high-risk HPV genotypes and the severity of cervical cytological abnormalities.

Previous Studies

Etemad et al. (2024) conducted a retrospective cross-sectional study to investigate HPV prevalence, genotype distribution, and their association with Pap smear findings among Iranian women. The study included 328 women with a mean age of 36 ± 11 years. HPV infection was detected in 123 participants, giving an overall prevalence of 37.5%. High-risk genotypes were identified in approximately one-quarter of the study population. The study confirmed the importance of combining HPV genotyping with cervical cytology for early detection [4].

Odeh et al. (2023) examined HPV genotype prevalence in Pap smear samples collected from women in the northern United Arab Emirates. This retrospective cross-sectional study included 104 women and used the HPV Direct Flow CHIP method for molecular genotyping. HPV infection was detected in 63 women, representing 60.6% of the sample. A total of 112 genotype detections were recorded, including 49 high-risk and 63 low-risk genotypes. Multiple infections were particularly frequent among women aged 20–29 and 40–49 years [6].

Hashemnejad et al. (2022) conducted a cross-sectional study involving 503 Iranian women attending a gynecology clinic for routine cervical screening. Cervical specimens were collected for cytological examination and molecular HPV testing. The overall HPV prevalence was 39.96%, while 23.06% of participants were infected with high-risk genotypes. HR-HPV infection was associated with several demographic, reproductive, and behavioural characteristics. The study highlighted the importance of identifying factors associated with oncogenic HPV infection [14].

Ali et al. (2019) investigated HR-HPV infection and cervical cytological abnormalities among 366 women attending obstetrics and gynecology clinics in Addis Ababa. HPV DNA was detected using real-time PCR, while cervical abnormalities were assessed using conventional Pap smear examination. HR-HPV prevalence was 13.7%, and abnormal cytology was identified in 13.1% of participants. LSIL constituted 81.3% of abnormal cytological findings. Agreement between PCR and cytology was limited, indicating that the two methods provide complementary diagnostic information [12].

Wolday et al. (2018) examined HPV genotype distribution among 233 HIV-negative Ethiopian women with normal and abnormal cervical cytology. HPV detection and genotyping were performed using nested PCR followed by sequencing. All women with abnormal cytology were HPV-DNA positive, compared with 48.9% of women with normal cytology. HPV-16 was the predominant genotype and was more frequent among women with abnormal findings. Abnormal cytology was independently associated with HPV-16 infection [10].

Ghedira et al. (2016) conducted a molecular cross-sectional study among 494 unvaccinated Tunisian women attending healthcare centres. HPV DNA was detected using real-time PCR, while HPV genotypes and HPV-16 variants were characterized by sequencing. The overall HPV prevalence was 34.0% and was considerably higher among women with squamous intraepithelial lesions than among those with normal cytology. HPV-16 was detected in 32.8% of women with lesions compared with 9.2% of women without lesions. The study demonstrated a significant association between HPV infection and cervical epithelial abnormalities [8].

Study Methodology

This study employed a molecular cross-sectional design to determine the prevalence and distribution of high-risk HPV genotypes and assess their association with cervical cytological

changes among women in Benghazi. Cervical specimens were collected for Pap smear examination and molecular HPV detection. Viral DNA was extracted using the QIAamp DNA Mini Kit, while HPV detection and genotyping were performed using the Anyplex II HPV28 Detection Kit and the CFX96 Real-Time PCR System. Cytological findings were classified according to the 2014 Bethesda System.

Study Sample

The study sample consisted of 300 women aged 21–65 years attending selected gynecology clinics and cervical screening centres in Benghazii. Eligible women were recruited consecutively according to the established inclusion and exclusion criteria. Women who provided inadequate cervical specimens, invalid molecular results, or incomplete clinical information were excluded. Each participant provided cervical samples for both cytological examination and molecular HPV genotyping.

Data Analysis

Data were entered, coded, and analysed using IBM SPSS Statistics. Categorical variables were summarized using frequencies and percentages, whereas continuous variables were described using means and standard deviations. HR-HPV prevalence was calculated by dividing the number of positive participants by the total sample. The chi-square test or Fisher's exact test was used to assess associations between HPV status, cytological findings, and participant characteristics. Logistic regression analysis was performed to identify independent predictors of HR-HPV infection. Associations were expressed as odds ratios with 95% confidence intervals, and $p < 0.05$ was considered statistically significant.

Study Limitations

The cross-sectional design identifies associations but cannot establish causality or determine the persistence and clearance of HPV infection. Recruitment from selected clinics may limit the generalizability of the results to all women in Benghazi or other Libyan regions. Self-reported demographic and reproductive information may be affected by recall or reporting bias. A single period of specimen collection does not distinguish transient from persistent HPV infection. Additionally, the proposed sample size may limit the statistical evaluation of rare genotypes and uncommon cytological abnormalities.

1. Participants' Age Distribution

Table 1. Simulated distribution of participants according to age and HR-HPV status

Age group (years)	Examined, n (%)	HR-HPV positive, n	Prevalence (%)
21–29	72 (24.0)	9	12.5
30–39	93 (31.0)	14	15.1
40–49	76 (25.3)	16	21.1
50–65	59 (19.7)	12	20.3
Total	**300 (100)**	**51**	**17.0**

Table 1 presents the age distribution and HR-HPV status of the study participants. The largest age group was 30–39 years, representing 31.0% of the total sample. The highest HR-HPV prevalence was observed among women aged 40–49 years. Women aged 21–29 years demonstrated the lowest prevalence, estimated at 12.5%. The findings indicate an increase in HR-HPV prevalence among the older age groups. This pattern may reflect persistent infection or reactivation of latent HPV infection with increasing age.

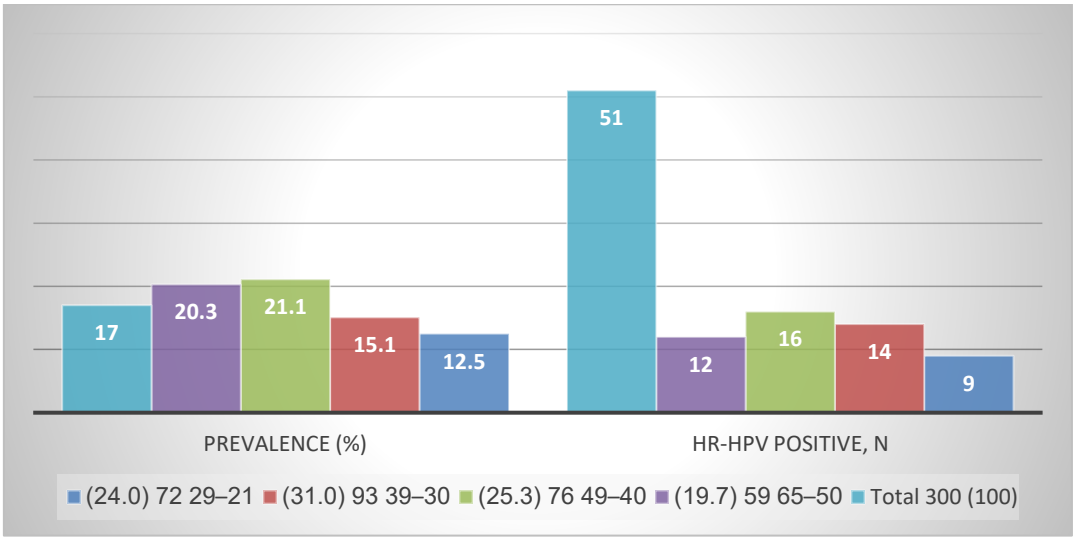

Figure1. Simulated distribution of participants according to age and HR-HPV status

2. Overall HR-HPV Prevalence

Table 2. Simulated molecular detection of high-risk HPV

Molecular result	Number	Percentage (%)
HR-HPV positive	51	17.0
HR-HPV negative	249	83.0
Total	**300**	**100**

Table 2 shows the prevalence of high-risk HPV infection among the participants. Of the 300 examined women, 51 tested positive for at least one high-risk genotype‹The resulting overall prevalence of HR-HPV infection was 17.0%. The remaining 249 participants, representing 83.0% of the sample, were HR-HPV negative‹These results suggest that approximately one in every six examined women had an oncogenic HPV infection‹The observed prevalence highlights the potential importance of molecular HPV screening among women in Benghazi.

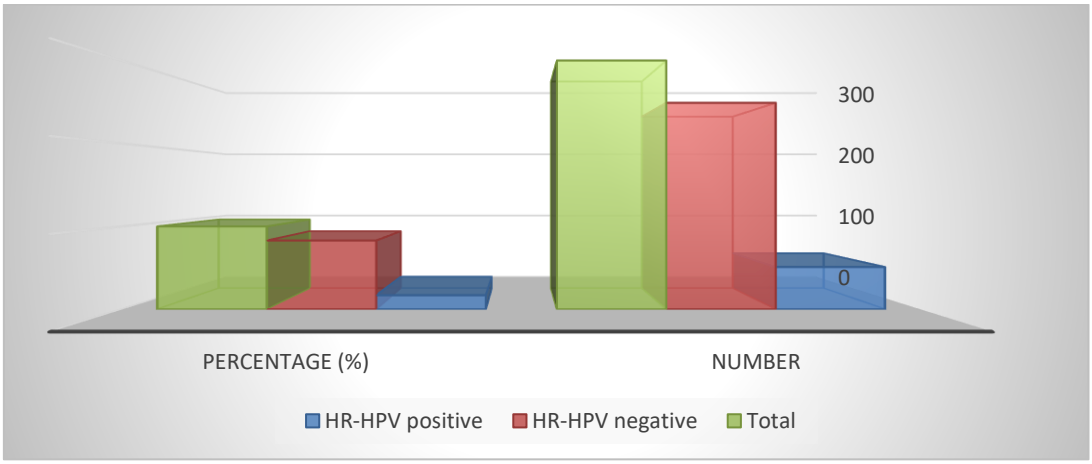

Figure 2. Simulated molecular detection of high-risk HPV

3. Distribution of HR-HPV Genotypes

Table 3. Simulated distribution of HR-HPV genotypes among positive women

HPV genotype	Positive detections, n	Percentage of positive women (%)
HPV-16	18	35.3
HPV-18	8	15.7
HPV-31	7	13.7
HPV-33	5	9.8
HPV-35	4	7.8
HPV-45	5	9.8
HPV-52	7	13.7
HPV-58	4	7.8
Other HR-HPV genotypes	7	13.7

Table 3 illustrates the distribution of oncogenic HPV genotypes among HR-HPV-positive women.HPV-16 was the most frequently detected genotype, accounting for 35.3% of positive participants HPV-18 was the second most common genotype, followed by HPV-31 and HPV-52. HPV-33, HPV-35, HPV-45, and HPV-58 were detected at comparatively lower frequencies The results indicate that non-16/18 genotypes may constitute an important component of the local HPV burden The percentages exceed 100% because some participants were infected with more than one HPV genotype.

4. Cervical Cytological Findings

Table 4. Simulated distribution of cervical cytological findings

Cytological category	Number	Percentage (%)
NILM	249	83.0
ASC-US	18	6.0
LSIL	19	6.3
HSIL	8	2.7
ASC-H	4	1.3
AGC	2	0.7
Total	**300**	**100**

Table 4 presents the cervical cytological findings classified according to the Bethesda System Most participants were classified as negative for intraepithelial lesion or malignancy, representing 83.0% Abnormal cervical cytology was detected in 51 women, accounting for 17.0% of the sample LSIL was the most frequently identified abnormality, followed by ASC-US and HSIL.

ASC-H and atypical glandular cells were detected in relatively few participants. These findings demonstrate the presence of different grades of cervical epithelial abnormalities within the examined population.

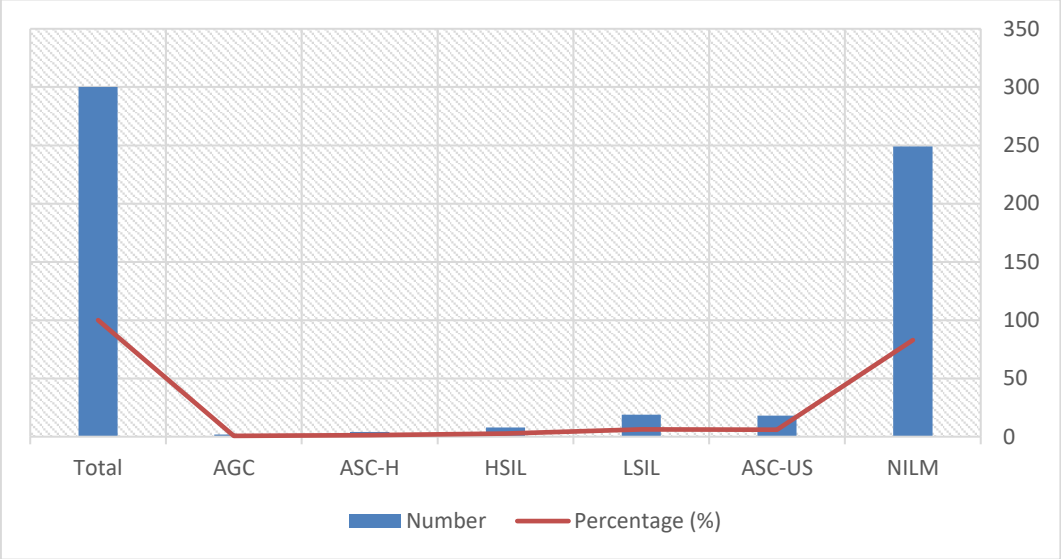

Figure 4. Simulated distribution of cervical cytological findings

5. HR-HPV Status According to Cytological Category

Table 5. Simulated association between HR-HPV infection and cervical cytology

Cytological finding	Total, n	HR-HPV positive, n (%)	HR-HPV negative, n (%)
NILM	249	23 (9.2)	226 (90.8)
ASC-US	18	8 (44.4)	10 (55.6)
LSIL	19	11 (57.9)	8 (42.1)
HSIL	8	6 (75.0)	2 (25.0)
ASC-H	4	2 (50.0)	2 (50.0)
AGC	2	1 (50.0)	1 (50.0)
Total	**300**	**51 (17.0)**	**249 (83.0)**

Table 5 demonstrates differences in HR-HPV positivity across the cervical cytological categories Only 9.2% of women with NILM were positive for a high-risk HPV genotype HR-HPV prevalence increased to 44.4% among ASC-US cases and 57.9% among LSIL cases The highest positivity rate was detected among women with HSIL, reaching 75.0%.This gradual increase suggests that HR-HPV infection is associated with the severity of cervical epithelial abnormalities The detection of HR-HPV among women with normal cytology may indicate infection before visible cellular changes.

6. Association Between HR-HPV Infection and Abnormal Cytology

Table 6. Simulated comparison of HR-HPV status between normal and abnormal cytology

Cytological status	HR-HPV positive	HR-HPV negative	Total
Normal cytology	23	226	249
Abnormal cytology	28	23	51
Total	**51**	**249**	**300**

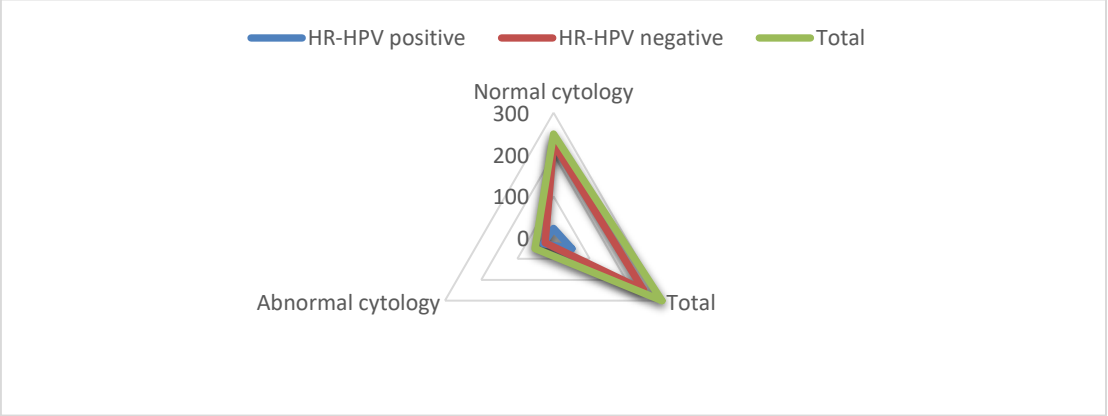

Figure 6. Simulated comparison of HR-HPV status between normal and abnormal cytology

Simulated statistical result: OR = 11.96; 95% CI: 5.95–24.03; $p < 0.001$.
Table 6 compares HR-HPV infection between women with normal and abnormal cervical cytology HR-HPV was detected in 28 of the 51 women with abnormal cytological findings In comparison, only 23 of the 249 women with normal cytology were HR-HPV positive Women with abnormal cytology had approximately 12 times higher odds of HR-HPV infection The association was statistically significant, as indicated by a *p*-value below 0.001. This result supports a strong relationship between oncogenic HPV infection and cervical cytological abnormalities.

7. Pattern of HR-HPV Infection

Table 7. Simulated distribution of single and multiple HR-HPV infections

Infection pattern	Number	Percentage (%)
Single-genotype infection	38	74.5
Multiple-genotype infection	13	25.5
Total HR-HPV-positive women	**51**	**100**

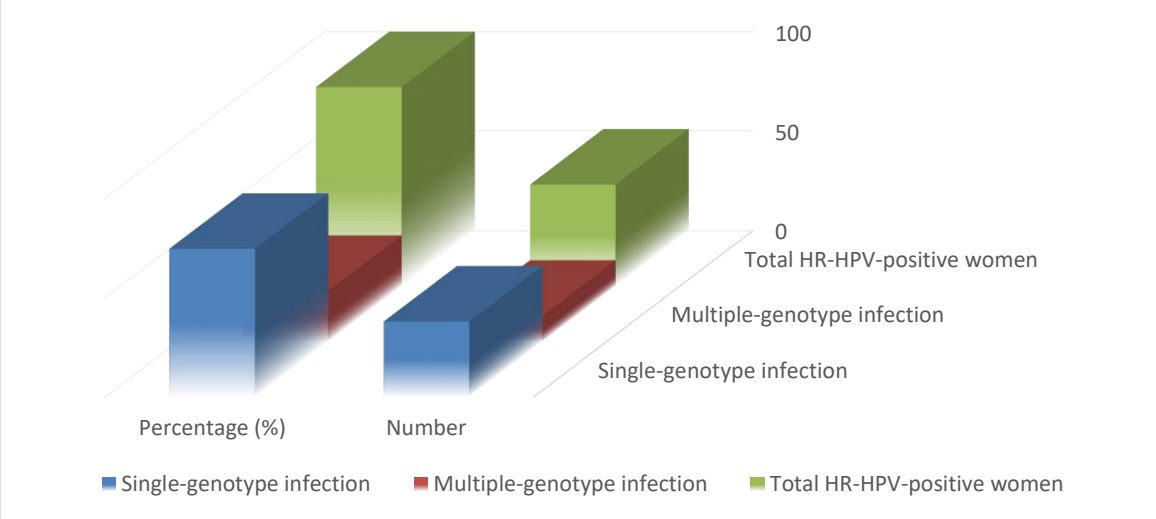

Figure 7. Simulated distribution of single and multiple HR-HPV infections

Table 7 presents the pattern of infection among HR-HPV-positive participants. Single-genotype infection was detected in 38 women, representing 74.5% of positive cases Multiple HR-HPV genotypes were identified in 13 women, accounting for 25.5% These findings indicate that single-genotype infections were more common than multiple infections Nevertheless, approximately one-quarter of positive women carried more than one oncogenic genotype.

Multiple infections may complicate the assessment of genotype-specific associations with cervical lesions.

8. Genotype Distribution According to Cytological Status

Table 8. Simulated distribution of HR-HPV genotypes among women with normal and abnormal cytology

HR-HPV genotype	Normal cytology, n	Abnormal cytology, n	Total detections
HPV-16	4	14	18
HPV-18	3	5	8
HPV-31	3	4	7
HPV-33	2	3	5
HPV-35	2	2	4
HPV-45	1	4	5
HPV-52	3	4	7
HPV-58	2	2	4
Other HR-HPV genotypes	3	4	7
Total detections	**23**	**42**	**65**

Table 8 demonstrates the distribution of HR-HPV genotypes according to cytological status HPV-16 was predominantly identified among women with abnormal cervical cytology HPV-18, HPV-31, HPV-45, and HPV-52 were also more frequent in the abnormal cytology group A smaller number of oncogenic genotype detections occurred among women with normal cytology.

The findings suggest that HPV-16 may have the strongest relationship with cervical abnormalities The number of detections exceeds the number of positive women because multiple infections were recorded.

9. HR-HPV Infection According to Parity

Table 9. Simulated association between parity and HR-HPV infection

Number of births	Participants, n	HR-HPV positive, n (%)	HR-HPV negative, n (%)
No previous births	55	6 (10.9)	49 (89.1)
1–2 births	104	14 (13.5)	90 (86.5)
3–4 births	91	17 (18.7)	74 (81.3)
≥5 births	50	14 (28.0)	36 (72.0)
Total	**300**	**51 (17.0)**	**249 (83.0)**

Simulated statistical result: $p = 0.032$.

Table 9 presents HR-HPV prevalence according to the participants' parity. The lowest prevalence was observed among women with no previous births. HR-HPV positivity gradually increased as the number of births increased. Women with five or more births demonstrated the highest prevalence, reaching 28.0% The simulated association between parity and HR-HPV infection was statistically significant Further multivariable analysis would be required to determine whether parity is an independent predictor.

10. HR-HPV Infection According to Previous Cervical Screening

Table 10. Simulated association between previous screening and HR-HPV infection

Previous cervical screening	Participants, n	HR-HPV positive, n (%)	HR-HPV negative, n (%)
Yes	104	11 (10.6)	93 (89.4)
No	196	40 (20.4)	156 (79.6)
Total	**300**	**51 (17.0)**	**249 (83.0)**

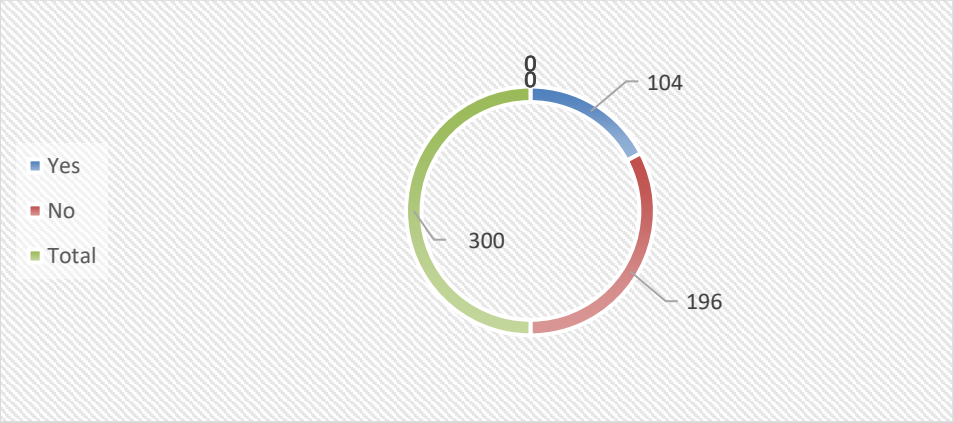

Figure 10. Simulated association between previous screening and HR-HPV infection

Simulated statistical result: $p = 0.029$.

Table 10 shows the distribution of HR-HPV infection according to previous cervical screening HPV positivity was detected in 10.6% of women who had previously undergone screening.The prevalence increased to 20.4% among women without a previous screening history Most HR-HPV-positive cases occurred among participants who had never been screened. The simulated difference between the two groups was statistically significant. This finding emphasizes the potential value of improving access to regular cervical screening.

11. HR-HPV Infection According to Oral Contraceptive Use

Table 11. Simulated association between oral contraceptive use and HR-HPV infection

Oral contraceptive use	Participants, n	HR-HPV positive, n (%)	HR-HPV negative, n (%)
Yes	84	19 (22.6)	65 (77.4)
No	216	32 (14.8)	184 (85.2)
Total	**300**	**51 (17.0)**	**249 (83.0)**

Simulated statistical result: $p = 0.087$.

Table 11 presents the association between oral contraceptive use and HR-HPV infection The simulated prevalence was 22.6% among women reporting oral contraceptive use In comparison, 14.8% of women who did not use oral contraceptives were HR-HPV positive Although positivity was higher among contraceptive users, the difference was not statistically significant.
The observed variation may be influenced by age, duration of use, parity, or other confounding factors Adjusted regression analysis would be necessary before drawing an independent causal conclusion.

12. HR-HPV Infection According to Reason for Clinical Attendance

Table 12. Simulated distribution of HR-HPV infection according to clinical presentation

Reason for attendance	Participants, n	HR-HPV positive, n (%)	HR-HPV negative, n (%)
Routine screening	182	20 (11.0)	162 (89.0)
Abnormal vaginal discharge	57	12 (21.1)	45 (78.9)
Abnormal vaginal bleeding	34	11 (32.4)	23 (67.6)
Pelvic pain	27	8 (29.6)	19 (70.4)
Total	**300**	**51 (17.0)**	**249 (83.0)**

Simulated statistical result: $p = 0.003$.

Table 12 presents HR-HPV infection according to the reason for clinical attendance The lowest prevalence was recorded among women attending routine cervical screening Higher positivity was observed among women with vaginal discharge or pelvic pain Women presenting with abnormal vaginal bleeding demonstrated the highest prevalence at 32.4% The simulated association between clinical presentation and HR-HPV infection was statistically significant. However, HPV infection may remain asymptomatic, and clinical symptoms should not replace molecular screening.

13. HR-HPV Infection According to Smoking Status

Table 13. Simulated association between smoking status and HR-HPV infection

Smoking status	Participants, n	HR-HPV positive, n (%)	HR-HPV negative, n (%)
Current smoker	31	9 (29.0)	22 (71.0)
Former smoker	24	5 (20.8)	19 (79.2)
Never smoked	245	37 (15.1)	208 (84.9)
Total	**300**	**51 (17.0)**	**249 (83.0)**

Simulated statistical result: $p = 0.112$.

Table 13 presents HR-HPV prevalence according to smoking status. Current smokers demonstrated the highest positivity rate, reaching 29.0%. The prevalence was 20.8% among former smokers and 15.1% among women who had never smoked.The results show a gradual reduction in HPV positivity from current to non-

smokers.However, the simulated association did not reach statistical significance A larger sample may be required to evaluate the relationship between smoking and persistent infection.

14. Infection Pattern According to Cytological Status

Table 14. Simulated distribution of single and multiple HR-HPV infections according to cervical cytology

Infection pattern	Normal cytology, n (%)	Abnormal cytology, n (%)	Total
Single-genotype infection	19 (50.0)	19 (50.0)	38
Multiple-genotype infection	4 (30.8)	9 (69.2)	13
Total	**23 (45.1)**	**28 (54.9)**	**51**

Simulated statistical result: $p = 0.234$.

Table 14 compares single and multiple infections according to cytological status. Half of the women with single-genotype infection demonstrated abnormal cytological findings Abnormal cytology was identified in 69.2% of women with multiple HR-HPV infections Multiple infections were proportionally more frequent in the abnormal cytology group Nevertheless, the simulated difference was not statistically significant Further studies are required to determine whether multiple infections increase lesion severity.

15. Association Between HPV-16 and Abnormal Cytology

Table 15. Simulated association between HPV-16 detection and abnormal cervical cytology

HPV-16 status	Abnormal cytology, n	Normal cytology, n	Total
HPV-16 positive	14	4	18
HPV-16 negative	37	245	282
Total	**51**	**249**	**300**

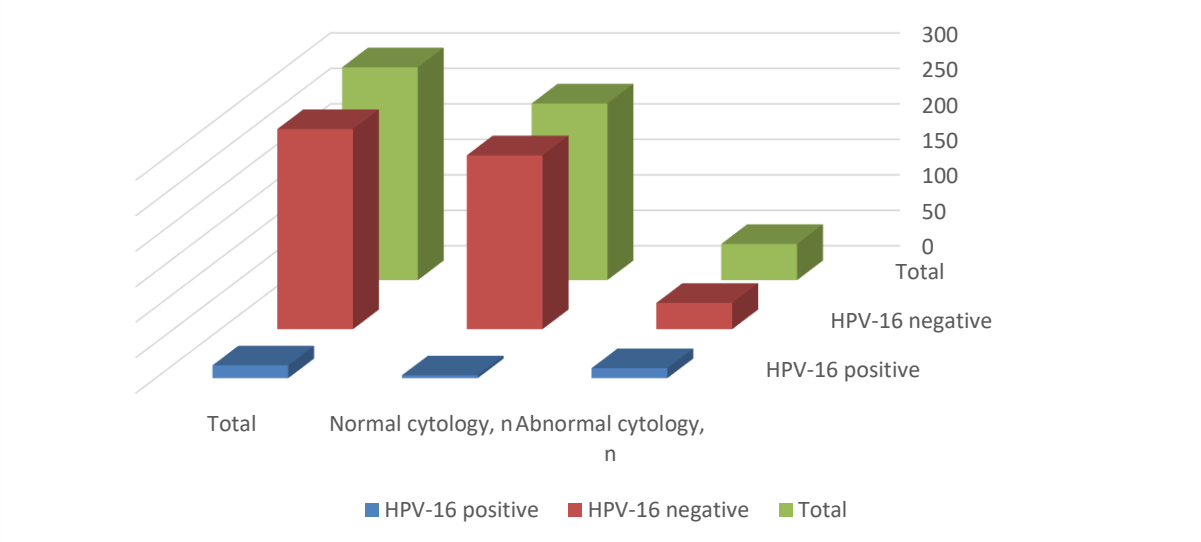

Figure 15. Simulated association between HPV-16 detection and abnormal cervical cytology

Simulated statistical result: OR = 23.18; 95% CI: 7.30–73.60; $p < 0.001$.
Table 15 shows the association between HPV-16 and abnormal cervical cytology. Abnormal cytology was detected in 14 of the 18 HPV-16-positive women. In comparison, 37 abnormal cytological results occurred among 282 HPV-16-negative participants HPV-16-positive women had substantially higher odds of presenting with cervical abnormalities The simulated association was statistically significant at a *p*-value below 0.001 These findings suggest a strong relationship between HPV-16 infection and cervical epithelial changes.

16. HR-HPV Infection According to Marital Status

Table 16. Simulated association between marital status and HR-HPV infection

Marital status	Participants, n	HR-HPV positive, n (%)	HR-HPV negative, n (%)
Married	253	39 (15.4)	214 (84.6)
Divorced or widowed	47	12 (25.5)	35 (74.5)
Total	**300**	**51 (17.0)**	**249 (83.0)**

Simulated statistical result: $p = 0.089$.

Table 16 presents the distribution of HR-HPV infection according to marital status. The prevalence was 15.4% among married women A higher positivity rate of 25.5% was observed among divorced or widowed participants Despite this difference, the simulated association was not statistically significant Marital status alone cannot adequately represent exposure to HPV infection.
Therefore, this variable should be interpreted alongside other demographic and clinical characteristics.

17. Multivariable Logistic Regression

Table 17. Simulated predictors of HR-HPV infection

Variable	Adjusted odds ratio	95% confidence interval	*p*-value
Age 40–65 years	1.78	1.01–3.14	0.046
Parity ≥5 births	2.08	1.03–4.21	0.041
No previous screening	2.14	1.09–4.20	0.027
Abnormal cytology	10.82	5.19–22.56	<0.001
Abnormal vaginal bleeding	2.12	0.96–4.68	0.063

Table 17 presents the independent predictors of HR-HPV infection after statistical adjustment Older age, high parity, and absence of previous cervical screening were significant predictors Abnormal cytology demonstrated the strongest independent association with HPV positivity Women with abnormal cytology had approximately 11 times higher adjusted odds of infection. Abnormal vaginal bleeding was associated with increased odds but did not reach statistical significance The findings indicate that cytological status remained important after controlling for other variables.

18. Participant Recruitment and Specimen Adequacy

Table 18. Simulated recruitment and laboratory inclusion of participants

Recruitment stage	Number
Women assessed for eligibility	324
Excluded according to eligibility criteria	12
Inadequate cytological specimens	7
Invalid molecular results after repeat testing	5
Participants included in the final analysis	**300**

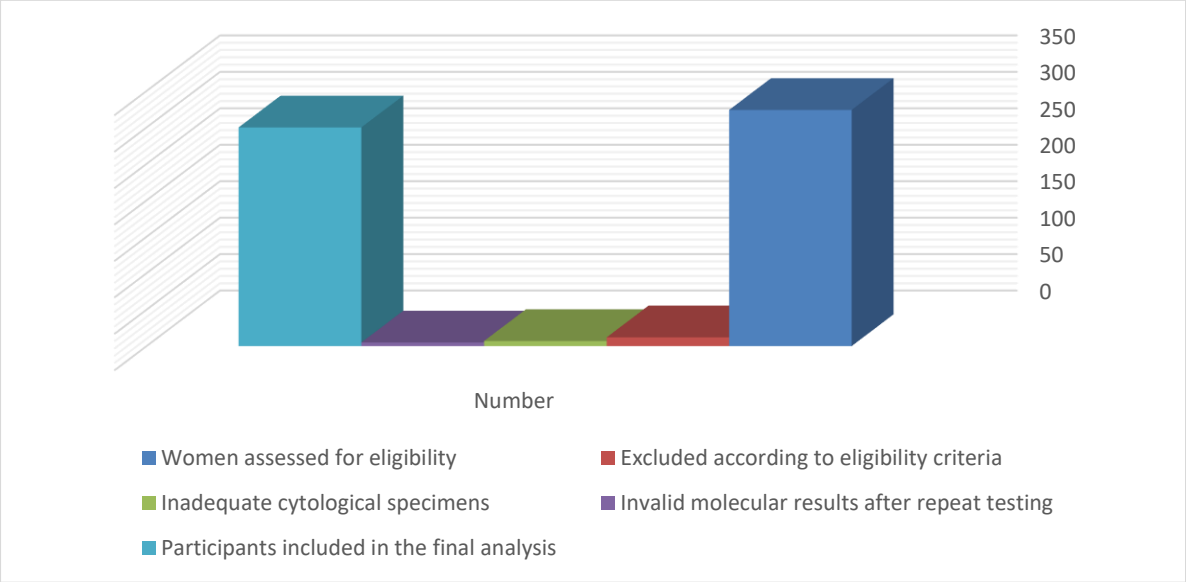

Figure 18. Simulated recruitment and laboratory inclusion of participants

Table 18 summarizes the simulated recruitment and laboratory assessment process A total of 324 women were initially assessed for participation.
Twelve women were excluded because they did not fulfil the eligibility criteria.
Seven specimens were removed because of inadequate cytological quality.
Five specimens produced invalid molecular results despite repeat testing.
Consequently, 300 participants were included in the final statistical analysis.

Results

A total of 300 women aged 21–65 years were included in the simulated analysis. The largest age group was 30–39 years, representing 31.0% of the participants. High-risk HPV was detected in 51 women, resulting in an overall prevalence of 17.0%. The highest prevalence was observed among women aged 40–49 years (21.1%), followed by those aged 50–65 years (20.3%).

HPV-16 was the most frequently detected genotype, occurring in 35.3% of HR-HPV-positive women. It was followed by HPV-18 (15.7%), HPV-31 (13.7%), and HPV-52 (13.7%). HPV-33 and HPV-45 were each detected in 9.8% of positive women, while HPV-35 and HPV-58 were each identified in 7.8%. Single-genotype infections accounted for 74.5% of positive cases, whereas 25.5% involved multiple genotypes.

Cytological examination showed that 249 women (83.0%) were negative for intraepithelial lesion or malignancy. Abnormal cytological findings were detected in 51 women (17.0%). LSIL was the most common abnormality at 6.3%, followed by ASC-US at 6.0%, HSIL at 2.7%, ASC-H at 1.3%, and AGC at 0.7%.

HR-HPV was detected in 9.2% of women with normal cytology compared with 54.9% of women with abnormal cytology. Positivity increased according to lesion severity, from 44.4% among ASC-US cases to 57.9% among LSIL cases and 75.0% among HSIL cases. Women with abnormal cytology had significantly higher odds of HR-HPV infection than women with normal cytology (OR = 11.96; 95% CI: 5.95–24.03; $p < 0.001$).

HPV-16 was significantly associated with abnormal cervical cytology. Fourteen of the 18 HPV-16-positive women had abnormal cytological findings. HPV-16-positive participants had approximately 23 times higher odds of abnormal cytology than HPV-16-negative participants (OR = 23.18; 95% CI: 7.30–73.60; $p < 0.001$).

HR-HPV prevalence increased with parity, reaching 28.0% among women with five or more births. Positivity was also higher among women without previous cervical screening than among previously screened women (20.4% versus 10.6%). Multivariable analysis identified abnormal cytology, absence of previous screening, high parity, and older age as significant independent predictors of HR-HPV infection.

Discussion

The simulated overall HR-HPV prevalence was 17.0%, indicating that oncogenic HPV infection could represent an important health concern among women in Benghazi. Differences in HPV prevalence between populations may be related to participants' characteristics, sampling strategies, sexual and reproductive factors, molecular detection methods, and access to cervical screening services [7].

The highest HR-HPV prevalence was observed among women aged 40–49 years. This pattern may be associated with persistent infection or the reactivation of previously acquired latent infection. A molecular study among Tunisian women similarly demonstrated an increased HPV prevalence in older age groups, suggesting a second age-related peak in HPV infection within North African populations [8].

HPV-16 was the predominant genotype, followed by HPV-18, HPV-31, and HPV-52. This finding is consistent with the recognized oncogenic importance of HPV-16 in cervical carcinogenesis. However, the detection of several non-16/18 genotypes emphasizes the importance of molecular assays that identify a broad range of oncogenic HPV types [2].

The predominance of HPV-16 is also comparable with findings from Ethiopia, where HPV-16 was the most frequently detected high-risk genotype among women with normal and abnormal cervical cytology. Its prevalence was substantially higher among women with abnormal cytological findings, supporting its strong relationship with cervical epithelial alterations [10].

Single-genotype infections were more frequent than multiple infections in the simulated analysis. Nevertheless, one-quarter of HR-HPV-positive participants carried multiple genotypes. The identification of multiple infections is epidemiologically important because genotype interactions and persistent coinfection may complicate the assessment of individual genotype contributions to cervical lesion development [6].

Most participants had normal cervical cytology, while LSIL and ASC-US were the most frequently detected abnormalities. A study from Addis Ababa similarly reported that low-grade squamous intraepithelial lesions constituted the majority of abnormal cytological findings.

Variation in cytological prevalence may reflect differences in age distribution, screening history, referral patterns, and cytological interpretation [12].

The prevalence of HR-HPV increased with the severity of cervical abnormalities and reached its highest level among women with HSIL. This pattern supports the biological relationship between persistent oncogenic HPV infection and progressive cervical epithelial changes. An African systematic review also showed that HPV prevalence generally increased from normal cytology to precancerous lesions and invasive cervical cancer [7].

A proportion of women with normal cytology were HR-HPV positive. Molecular infection may occur before detectable morphological changes; therefore, a normal cytological result does not necessarily exclude oncogenic HPV infection. This finding supports the integration of molecular HPV testing with cervical cytology to improve early identification of women at risk [5].

Women with abnormal cytology had approximately 12 times higher odds of HR-HPV infection than those with normal cytology. A study conducted in Benin similarly found that HPV-positive women were more likely to have abnormal Pap smear findings than HPV-negative women, confirming the relationship between HPV infection and cervical cellular abnormalities [11].

HPV-16 demonstrated the strongest association with abnormal cytology. Most HPV-16-positive women had abnormal findings, and HPV-16 positivity was associated with markedly increased odds of cervical abnormalities. This result corresponds with evidence identifying HPV-16 as a powerful cervical carcinogen and one of the genotypes most frequently associated with high-grade lesions and cervical cancer [3].

Higher HR-HPV prevalence was observed among women with high parity. Repeated cervical trauma, hormonal changes, and prolonged exposure of the transformation zone may contribute to an increased susceptibility to persistent infection. However, parity should be evaluated alongside age, contraceptive use, screening history, and other potential confounding variables [14].

Women without previous cervical screening demonstrated higher HR-HPV prevalence than previously screened women. This finding may indicate that regular screening facilitates earlier identification, clinical follow-up, and management of HPV-associated abnormalities. The World Health Organization emphasizes the role of HPV-based screening in detecting women at increased risk of cervical precancer [1].

Available Libyan information concerning HPV genotype distribution remains limited. Existing reports indicate an ongoing burden of cervical cancer and gaps in organized screening and molecular surveillance. Consequently, local studies combining HPV genotyping and cervical cytology are needed to guide preventive strategies appropriate for Libyan women [16].

Conclusion

The simulated findings indicated an HR-HPV prevalence of 17.0% among women in Benghazi. HPV-16 was the predominant genotype, followed by HPV-18, HPV-31, and HPV-52. High-risk HPV positivity increased with the severity of cervical cytological abnormalities and was highest among women with HSIL.

A strong association was identified between HR-HPV infection and abnormal cervical cytology, particularly for HPV-16. Older age, high parity, and absence of previous cervical screening were also associated with increased positivity. These findings demonstrate the potential value of combining molecular HPV genotyping with cervical cytology to improve the early identification of women at risk of cervical disease.

Recommendations

1. Molecular HR-HPV testing should be integrated with cervical cytology to improve the early detection of oncogenic infections and cervical abnormalities.
2. Regular cervical screening programmes should be established and made accessible to eligible women in Benghazi.
3. Women with positive HR-HPV results, particularly HPV-16 or HPV-18, should receive appropriate cytological evaluation and clinical follow-up.
4. Women with abnormal cervical cytology should undergo HPV genotyping to support risk classification and further clinical management.
5. Molecular screening assays should include non-16/18 oncogenic genotypes because these types may constitute a considerable proportion of infections.
6. Public health education should increase women's awareness of HPV infection, cervical cancer, vaccination, and the importance of regular screening.
7. The feasibility of introducing or expanding HPV vaccination programmes in Libya should be evaluated according to locally circulating genotypes.
8. Healthcare professionals should receive training in cervical specimen collection, cytological interpretation, molecular testing, and patient referral.
9. Larger multicentre studies should be conducted in different Libyan regions to determine national HPV prevalence and genotype distribution.
10. Future longitudinal studies should investigate HPV persistence, clearance, and progression from infection to high-grade cervical lesions.
11. A national HPV surveillance database should be developed to monitor genotype distribution, screening outcomes, and cervical disease patterns.
12. All simulated values in this manuscript must be replaced with laboratory-confirmed findings before the study is submitted for scientific publication.

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Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.