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Distribution of Human Papillomavirus in Precancerous and Cancerous Cervical Neoplasia in Tunisian Women

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Abstract

Background

High-risk human papillomavirus (HR-HPV) are responsible for cervical cancer (CC), that is a major health problem worldwide and the second most prevalent gynecological cancer among Tunisian women. Preventive tools against CC are based on screening and prophylactic vaccines. Improving the preventive strategies as well as the therapeutic algorithms needs the understanding of HPV distribution in cervical lesions in Tunisian women.

Methods

A total of 200 formalin-fixed paraffine embedded biopsies were collected in our study. DNA was extracted using Qiagen Mini prep kit. DNA quality was controlled by Beta Globin PCR. Only positive samples for Beta Globin test were used. HPV detection was performed by a nested PCR using PYGMY and GP5+/6+ primers. Genotyping was performed by Reverse Line hybridization using 31 probes.

Results

The mean age of participants was 38.97 years and 54.5% were over 40 years. Cervical neoplasia distribution according to age showed that CINII/CINIII was observed in women over 30 years old. All samples were positive for Beta Globin PCR. Global HPV prevalence in cervical lesions was 83% (166/200). HPV was present in 65% in CINI, 82% in CINII/CINIII and 85% in CC. HR-HPV was statically associated with cervical intraepithelial neoplasia ($p < 10^{-3}$). HR-HPV distribution according to lesion grade and cervical cancer showed that HPV16 and HPV18 were present in all lesions. For CINII/CINIII, HPV 35 (33,33%) was the most detected type, followed by HPV18 (28,6%), HPV 45 (25%) and HPV 16 (21,7%). HPV 45(62%), HPV 18 (47%) were the most detected in CC. HPV58, 59, 66 were only detected in CC and associated with HPV45,18 and HPV16. HPV39, 31, 33, 52, 56, 68 and HPV70 was associated only with CINI.

Conclusions

These findings show that HR-HPV represents 54,6 % of all infections in all cervical lesions. Five HR-HPV (35, 18, 45,16, 51) were detected in CINII/CINIII with a high incidence of HPV 35, 18, 45, and 16 that are included in the proposed vaccines. These findings give useful information for vaccine implementation consideration as well as personalized decision algorithms of intra-epithelial cervical lesions in Tunisian women.

Background

Cervical cancer (CC) is the second gynecological cancer in women worldwide [1, 2] with a global annual incidence of 569,847 in 2018, and 311,365 annual death rate [3]. In Northern Africa, the incidence of CC is

7652 cases with 5243 deaths annually [3]. Among Tunisian women, CC ranks fourth after breast, lung and colorectal cancer [4], with 299 new cases each year and about 200 deaths per year[5–7].

Pap smear screening has not been successful for preventing CC in Tunisian women. It seems necessary to offer new epidemiological considerations that could possibly include vaccine implementation and HPV testing in a global efficient preventive strategy.

It is well known that HPV is the first causal agent of CC [8]. About 201 different HPV types have been identified. More than 40 types infect the mucous membranes and have been classified into Low-risk (LR-HPV) and High-risk (HR-HPV) depending on their ability to induce malignant progression [9, 10]. Most HPV infections are cleared with the immune system response. Cervical intraepithelial neoplasia (CIN) is a progressive pathophysiological process with two different pathways [11]. The first one is a spontaneous regression of the cervical lesions. The second is the persistence of the infection with a HR-HPV that can progress to high grade intraepithelial neoplasia (CINII, CINIII) and CC in 8 to 12 years [10, 12, 13].

As HPV infection prevalence and genotype distribution is known to vary significantly in different countries and world regions [14, 15] their investigation in specific areas provides a scientific basis for the measures and methods an efficient preventive and therapeutic strategy including vaccine implementation and HPV testing. Prevalence and distribution of HPV types in different stages of cervical intraepithelial neoplasia has not yet been studied in Tunisia to our knowledge. This work aims to provide Tunisian scientific data that can be useful for an efficient management of cervical intra-epithelial lesions in Tunisian women.

Methods

Population study

This is a retrospective study including 200 women aged between 19 ~ 59 years old with cervical intraepithelial neoplasia diagnosed on biopsies. Samples are paraffin embedded blocks that were collected between 2016 and 2019 in the department of pathology of Pasteur Institute of Tunis. Cervical intraepithelial neoplasia was classified according to the Bethesda system [16] as bellow (Table 1):

Table 1
Samples number according to cervical intraepithelial neoplasia.

| Cervical lesion | CINI | CINII/CINIII | CC | Total |
|-----------------|------|--------------|----|-------|
| Number | 82 | 92 | 26 | 200 |

DNA Extraction

Paraffin blocks were cut into 10µm thin preparations. The rewashing step was performed by three washing baths in Xylen (incubation at 65°C for 15 min) followed by four washings with ethanol with

decreasing concentrations. DNA was then extracted by Qiagen Mini prep kit according to the manufacturer's instructions. The DNA quality was evaluated by a beta-globin test using specific primers PC04/GH20. DNA purity was measured by Nanodrop spectrophotometer.

HPV DNA detection and typing

HPV detection used a nested PCR with PGMY 09/11 primers for the first PCR and biotinylated GP5+/GP6 + for the second PCR. PCR was performed with a positive control (plasmid with HPV) and a negative control. The first PCR cycling parameters were composed of 1min initial denaturation at 94°C, followed by 30 amplification cycles of 30s at 94°C, 2 min at 54°C and 1 min at 72°C. This reaction was followed by a nested PCR using 10µl of the PGMY PCR product in a reaction mixture containing 50µmol of GP5+/GP6 + primers, 3 mM Mgcl₂, 1.5mM each of the dNTP, 1U of Taq DNA polymerase and 5µl of Taq DNA polymerase buffer. The PCR cycling parameters contained a 4 min initial denaturation followed by 40 cycles of 30s at 94°C, 1 min at 50°C, 1min30s at 72°C and a final extension step for 10 min at 72°C. Molecular genotyping was performed on samples that had positive signal amplification with the nested PCR and used a Reverse blotting hybridization as described in the Human Papillomavirus Laboratory Manual published by the WHO (World Health Organization). 15µl denatured PCR products could hybridize with primers probes specific for 31 HPV types (HPV6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68, 69, 70, 73, 82, 83 and 84), immobilized on a Biotodyne C membrane using a Miniblotter MN45. The hybridized DNA was detected using streptavidin peroxidase and ECL.

Statistical study

Statistical analyses used the Statistical Package for the Social Sciences (SPSS) software version 20.0 (IBM, Somers, NY, USA). Pearson's chi-square test was calculated to associate HPV detection and HPV genotypes with different variables. P values < 0.05 were considered statistically significant.

Ethical considerations

The study was approved by the Ethical committee of Institut Pasteur de Tunis (2018/03/LAPHEIPT) and conducted with good clinical practice, ensuring confidentiality and anonymity.

Results

1. Population study

A total of 200 samples were collected for this study from women aged between 19 and 59 years old. The mean age of participants was 38.97 years and 54.5% were over 40 years.

Cervical neoplasia distribution according to age showed that CINII/CINIII were observed in women over 30 years old (Table 2). No statistical significance between age and lesions was found ($p > 0.05$).

Table 2
Intraepithelial cervical lesions distribution according to age group

| | | CINI | CINII/CINIII | Cervical cancer | Total |
|------------------|----------------|-------|--------------|-----------------|--------|
| Age group | <=30 | 31.6% | 57.9% | 10.5% | 100.0% |
| |]30–40] | 17.1% | 63.4% | 19.5% | 100.0% |
| |]40–50] | 8.3% | 61.1% | 30.6% | 100.0% |
| | > 50 | 0.0% | 66.7% | 33.3% | 100.0% |

CINI: Cervical intraepithelial neoplasia I, CINII: Cervical intraepithelial neoplasia grade II, CINIII: Cervical intraepithelial neoplasia grade III.

2. Global HPV prevalence

All samples were positive for the β globin test. HPV detection and typing were performed for 200 samples. HPV was detected in 166 samples leading to a global prevalence of 83%.

The mean age of HPV positive women was 39.56 years old. The most prevalent age group within HPV positive women was < 30 and > 50 (Fig. 1) without statistical significance ($p > 0.05$).

HPV was present at the rate of 65% in CINI. For CINII/CIN III and CC HPV prevalence was respectively 82% and 85% (Fig. 2).

3. HPV genotype distribution

A total of 22 genotypes was identified and were classified into HR-HPV types and LR-HPV (Fig. 3).

Multiple and single infections were detected respectively in 43% and 57% of cases. No statistical association between infection type and age group or cervical intraepithelial neoplasia (Table 3) was found. HR-HPV and LR-HPV prevalence were respectively 54.6% and 65.4%.

Table 3
Infection type distribution according to age group.

| Age group | Infection type | | p | |
|------------------------------------|--------------------|------------------|-------|------|
| | Multiple infection | Single Infection | | |
| < 30 | 63.2% | 36.8% | 0.8 | |
| 30–39 | 56.1% | 43.9% | | |
| 40–50 | 52.8% | 47.2% | | |
| > 50 | 50.0% | 50.0% | | |
| Cervical intraepithelial neoplasia | CINI | 13.3% | 20% | 0.16 |
| | CINII/CINIII | 68.1% | 61.7% | |
| | CC | 25% | 11.4% | |

CINI: Cervical intraepithelial neoplasia I, CINII: Cervical intraepithelial neoplasia II, CINIII: Cervical intraepithelial neoplasia grade III, p: p value for the association between HPV type infection, age and cervical neoplasia.

The prevalence of HR-HPV and LR-HPV was respectively 54.6% and 65.4%. HR-HPV was associated with cervical intraepithelial neoplasia ($p < 10^{-3}$).

The most prevalent LR-HPV type was HPV 6 followed by HPV11, 42, 40, 43 and HPV 54 (Fig. 3). LR-HPV were prevalent in the age group (30–40) (Fig. 1).

The predominant HR-HPV were HPV 16, 18, 53, 35, 45, 39, 51, 52, 33, 59, 68, 70, 66 and HPV 56. HR-HPV were observed mostly in women in the age group (40–50) (Fig. 1).

4. HR HPV- types distribution according to cervical intraepithelial neoplasia

HR-HPV distribution according to CIN and CC showed that HPV 16 and HPV18 were present in all grades (Table 4.). They were associated with HPV58, HPV59, HPV66 which were present only in CC. HPV 39, 31, 33, 52, 56, 68 and HPV70 were detected only in CINI (Table 4). For CINII/CINIII, HPV 35 (33,33%) was the most detected type, followed by HPV18 (28,6%), HPV 45 (25%) and HPV16 (21,7%). HPV 45(62%), HPV 18 (47%) and HPV16 (26,1%) were the most detected in CC.

Table 4
Distribution of HR-HPV types according to cervical intraepithelial neoplasia

| Cervical intraepithelial neoplasia | | | |
|------------------------------------|--------|--------|--------------|
| HPV Type | CC | CINI | CINII/CINIII |
| HPV16 | 26.1% | 52.2% | 21.7% |
| HPV18 | 47.6% | 23.8% | 28.6% |
| HPV31 | 0.0% | 100.0% | 0.0% |
| HPV33 | 0.0% | 100.0% | 0.0% |
| HPV35 | 33.3% | 33.3% | 33.3% |
| HPV39 | 0.0% | 100.0% | 0.0% |
| HPV45 | 62.5% | 12.5% | 25.0% |
| HPV51 | 0.0% | 85.7% | 14.3% |
| HPV52 | 0.0% | 100.0% | 0.0% |
| HPV53 | 33.3% | 66.7% | 0.0% |
| HPV56 | 0.0% | 100.0% | 0.0% |
| HPV58 | 100.0% | 0.0% | 0.0% |
| HPV59 | 100.0% | 0.0% | 0.0% |
| HPV66 | 100.0% | 0.0% | 0.0% |
| HPV68 | 0.0% | 100.0% | 0.0% |
| HPV70 | 0.0% | 100.0% | 0.0% |

CINI: Cervical intraepithelial neoplasia I, CINII: Cervical intraepithelial neoplasia II, CINIII: Cervical intraepithelial neoplasia III.

Discussion

Cervical cancer is a multistep disease and persistent infection with HR-HPV is the major cause of intraepithelial neoplasia and cervical cancer. An efficient preventive and therapeutic strategy including vaccine consideration and HPV testing needs to determine the HPV genotypes distribution in different cervical lesions. Previous Tunisian studies has been conducted on cervical cancer or women with normal pap smear [4, 17]. To our Knowledge, there is few epidemiologic data concerning HPV genotypes in cervical neoplasia from Tunisia. Our study provides results about HPV genotype distribution in different stages of cervical lesions that could be useful for a global preventive strategy of CC and therapeutic algorithms for CIN including vaccine implementation and HPV testing.

The mean age of participants in our study was 38.97 years and 54.5% were over 40 years. The mean age of HPV positive women was 39.56 years old. In our study population we found that there are two age peaks of HPV infection prevalence in women with cervical neoplasia: under 30 years old and over 50 years old. The infection rate during these ages was significantly higher than in the other age groups, suggesting a “U”-shaped infection. In most studies the highest peak is seen in younger women (under 25 years old) then a decreasing trend with age is observed and another maximum peak around 50 years old [18, 19].

In our population study, global HPV prevalence in cervical lesions was 83%. Our data showed that 65% of patients with CIN I, 82% with CIN II/CIN III and 85% with CC were HPV positive. These results are concordant with other studies conducted in center of Tunisia [1, 7–9, [20] which shows that global prevalence is 73,6%; 84% in CIN I and 83,9% in CC. Our results are also consistent with those in most other studies in the word [21, 22]. A recent study in Morocco showed that HPV infection was 92,5% in CC [23]. The HPV positivity in Longnan-China women was 74,6% in CIN I, 87,5% in CIN II/CIN III and 89,05% in CC [24].

We can conclude that despite the small size of our subgroup, our results are consistent with most reports, which prove the performance of our method of HPV detection and genotyping. Our data reflects the real association pattern between HPV infection and cervical intraepithelial neoplasia subgroup.

Of all HPV positive samples, there was a great proportion of patients with multiple HPV types (43%), but without correlation between coinfection and age. This is not consistent with other studies [25], which showed age-specific prevalence of multiple HPV infections.

In our series HR-HPV was associated with cervical intraepithelial neoplasia ($p < 10^{-3}$). The predominant HR-HPV was HPV 16, 18, 53, 35, 45, 39, 51, 52, 33, 59, 68, 70, 66, 56. HR-HPV were observed in lower age < 30-year group and mostly in women in the age group (40–50). In a study of Guardado- Esatrada M, 2014 [26], it is reported that in CC Mexican patients, the first peak was found in the youngest women 35 \leq years, the second peak was at 61–65 years and the mean ages of the patients singly infected with HPV16, HPV 18, HPV 45 and HPV 39 were at least 5 years lower than the patients singly or doubly infected with other HPV types and HPV 16, 18, 45 and 39 trends decrease [26]. In Karrollina Aro et al, 2019 study, it is reported that in CIN II/CIN III HPV16 and HPV18 are more common in younger women under 30 years old then over 45 years old [25].

CIN I may appear within 4 months after HPV infection and if associated with certain HPV genotypes, could progress to CIN II, CIN III and cervical cancer. Assessing HPV genotype among CIN I is therefore suitable to identify women at risk of progression. Our results indicate that the most frequent HPV genotypes in CIN I in order of decreasing prevalence were HPV31, 33, 39, 52, 56, 68, 70, 51 and HPV 53. The meta-analysis [27] of Guan P et al, 2012 reported that HPV 35, 39, 51, 56 and 68 were present in low and High grade lesions but were low in CC, which prove the low carcinogenic potential of this types.

In our series the most prevalent genotypes in CIN II/CIN III were HPV 35, 18, 45 and HPV16. In CC, HPV45, 18, 35 and HPV16 were predominant. HPV 16 and HPV18 were detected in all lesions. Our results are

concordant with the meta-analyses of Clifford GM. et al showing that in the fifteen high risk HPV genotypes, HPV16 and 18 were found in approximately 70% of cervical cancer worldwide [28]. We also demonstrate that HPV16, 18 and 45 were detected in CC. HPV45 is mostly present in CINII/CINIII and CC.

In fact, HPV 45 seems to be more frequent in Africa and less in European, American and Asian population [22, 24, 27]. In Europe, HPV16 and HPV18 are the most common types. HPV 58 is the most prevalent in Asia. In Saudi Arabia, the most common genotypes in CC were : HPV16, 18, 31, 45, 56, 59 and HPV73 [29]. In A worldwide meta-analysis of over 115 000 HPV-positive women HPV16, HPV18 were the most prevalent HPV types in cervical lesions, while HPV52 and HPV58 were most prevalent in East Asian women [14]. Other study in India describes HPV16, 18 and 58 as the most common types [15]. The Nano-valent vaccine seems to include all world regions HPV distribution [22, 24].

Our findings show that HPV16, 18, 45, 35, 51,53, 58, 59 and 66 HPV types detected in CINII/III and CC play an important role in the development of cervical cancer neoplasia in Tunisian women. Our results are concordant with other studies in the world [7, 11, 16, 17]. We also demonstrated the increased prevalence of HPV18 associated with CC which is less frequently represented in precancerous lesions as CINI and CINII/III. The higher risk for developing CINIII reported for HPV 18 was described in the Meta-analysis study of Clifford GM, 2003 [28].

Conclusions

Our results offer the basis to identify efficient clinical management approaches with HPV testing to avert cervical cancer in Tunisia also aiming to reduce the cost-effectiveness of current clinical care that supports cervical cancer prevention. Screening modalities and strategies, as well as clinical management algorithms, will need to evolve with a rational integration of HPV vaccination and cervical screening.

In this Tunisian series HR-HPV prevalence increases from CINI to CINII/CINIII and CC. HPV 35, 18, 45, 16 are the predominant HR-HPV in women with high grade intraepithelial cervical neoplasia CINII/CINIII. These results are meaningful for HPV test screening as well as personalized early decision algorithms and treatment of HR-HPV infected Tunisian women and can improve preventing progression into cervical cancer.

Abbreviations

HPV: Human papillomavirus

HR: High risk

LR: Low risk

CINI: Cervical Intraepithelial Neoplasia I

CIN II:Cervical Intraepithelial Neoplasia II

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Figures

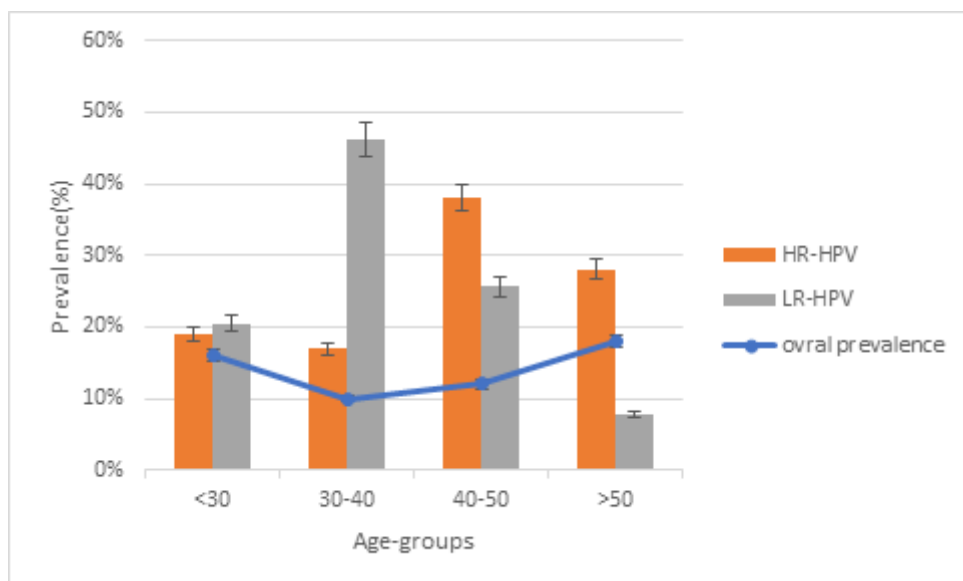


Figure 1

HPV prevalence distribution according to age groups

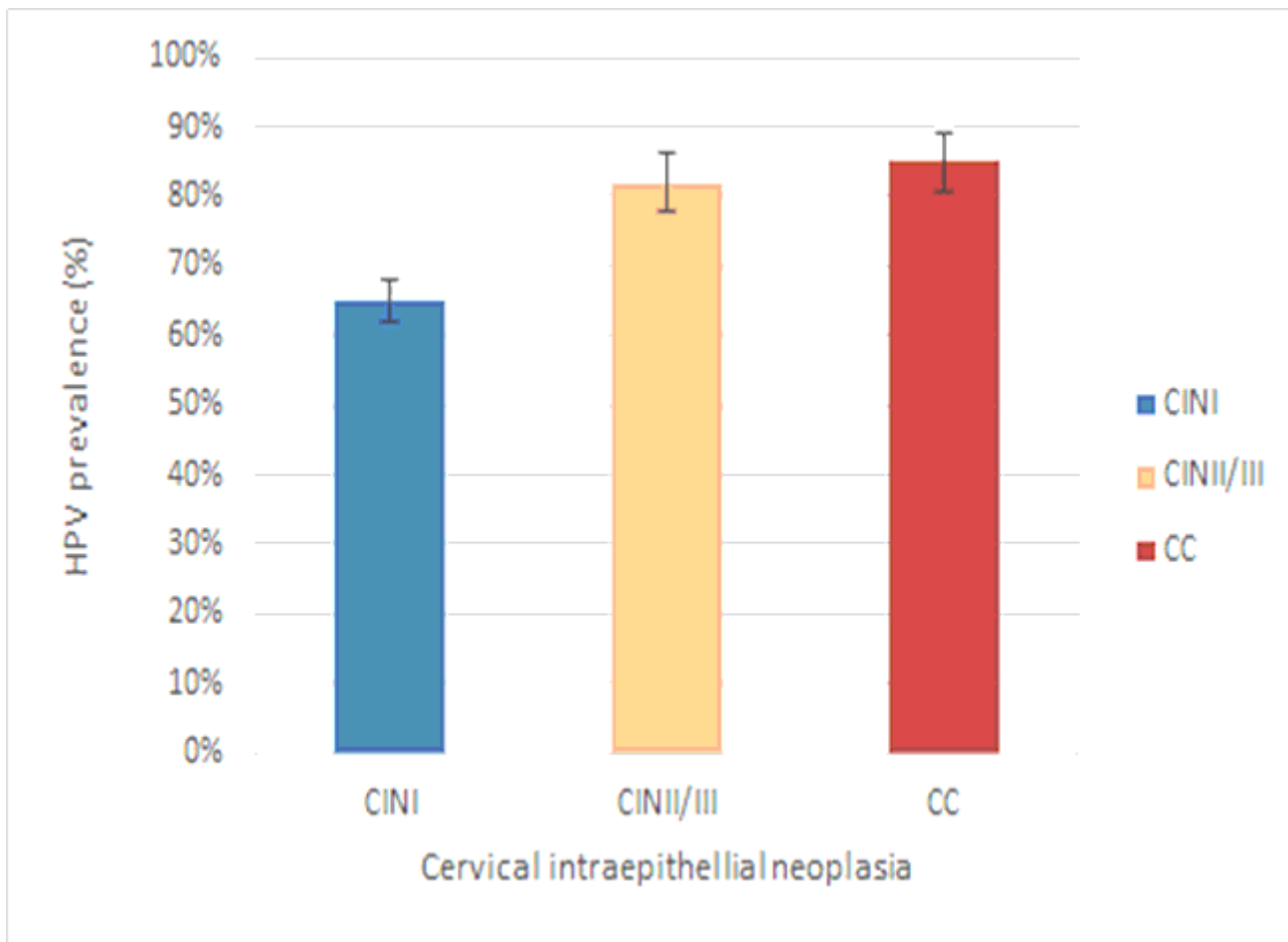


Figure 2

HPV prevalence according to the cytological statute

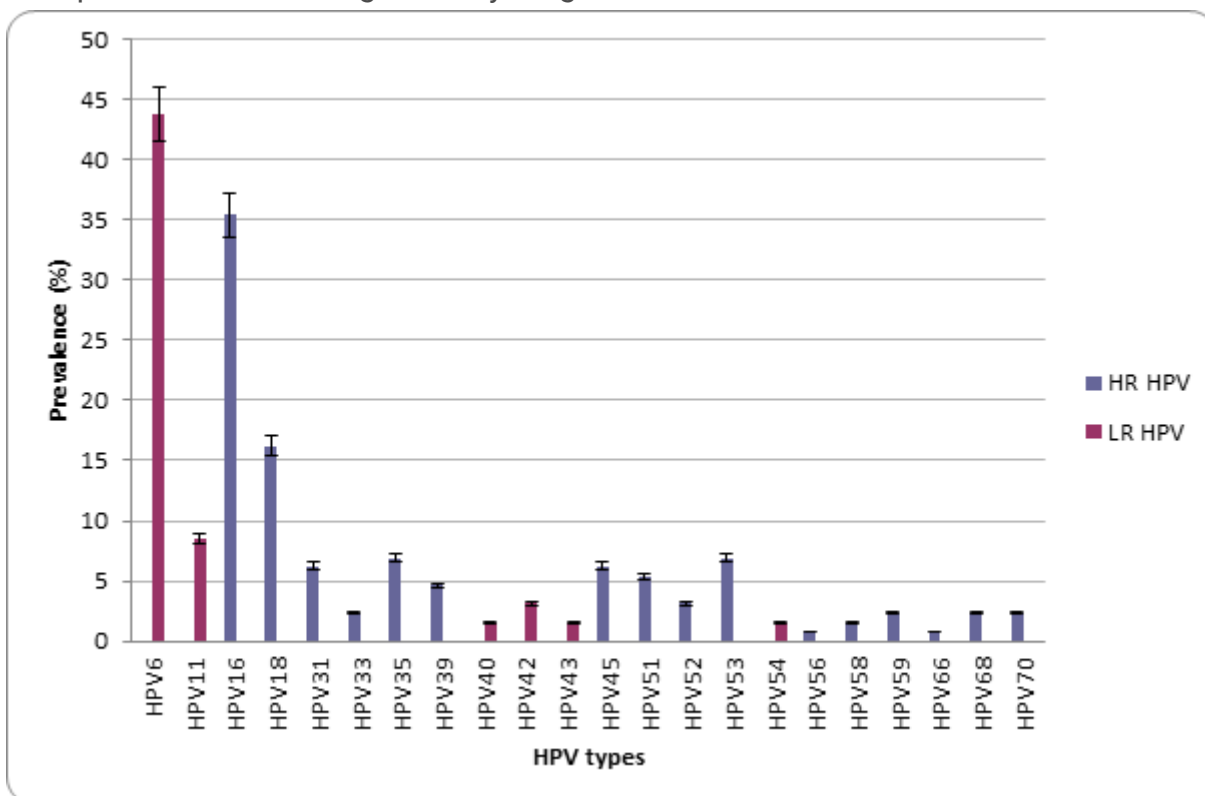


Figure 3

Distribution of HR-HPV and LR-HPV genotypes intraepithelial cervical neoplasia.