



Human Papillomavirus and Cervical Intra-Epithelial Neoplasia: Epidemiological and Cytological Study in Lubumbashi Women

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Abstract: The objective of this study is to show that the presence of koilocytosis on the cervical smear is the only possibility to detect Papillomavirus infection in cervical intra-epithelial neoplasia in women living in Lubumbashi. This is a cross-sectional analytical study on the data collected from the register of women who participated in the cervix lesion screening campaign organized in Lubumbashi in March 2012 by the Congolese League Against Cancer. A total of 545 women with cervical lesions have been selected. The following results were observed: the frequency of HPV infection is 35% in Lubumbashi women. The risk of developing intraepithelial neoplasia is 38.3 times higher in women infected with HPV compared to uninfected ones (OR = 38.3, 95% CI = 16.3-90.3). HPV is predominantly present in intraepithelial neoplasia (92.7%) and this regardless of their grade: 91.9% for low-grade neoplasia and 95% for high-grade neoplasia. HPV is found in both older women and older women, respectively in 45% of women aged less than 36 years and in 55% of women aged over 36 years old. This study shows that it is necessary to put in place adequate means for the detection of HPV in order to contribute to the fight against cervical cancer in Lubumbashi.

Keywords: Papillomavirus, Intra Epithelial Neoplasia, Cervix, Lubumbashi

1. Introduction

Papillomaviruses are a large family of small, non-enveloped DNA viruses with epithelial tropism and high resistance [1, 2]. Their genome is a circular DNA molecule of about 8,000 nucleotide pairs. They have in common a genetic organization characterized by the presence of eight genes, encoding proteins involved in the interaction with the host cell (E genes) or coding for constituents of the viral capsid (L genes) [2]. These genes are located downstream from a noncoding region, containing sequences regulating the replication and transcription of the viral genome [2]. Human papillomavirus (HPV) genital infection has two diametrically

opposite aspects, which are, on the one hand, the common sexually transmitted disease, very common, most often asymptomatic and transient, accompanied by acquired and effective immunity.; On the other hand, this infection is a disease inducing epithelial changes [3], which can lead to the development of cervical cancer and its precursor lesions as demonstrated by numerous clinical, epidemiological and molecular studies [4]. Women at risk for HPV infection are characterized by premature sexual life and multiple sexual partners or sexual promiscuity of their partner (s), suggesting the sexual transmission of a long-lasting [5]. This factor leads to cellular alteration in the form of koilocytosis, identifiable on cervical smears [2]. Koilocytosis are the stigma of papillomavirus replication in the cervix [6] and are frequently

associated with intra-epithelial neoplasia. The frequency of this virus on cervical smears varies from 25 to 50% in women aged 15 to 25 who are sexually active [2]. This prevalence decreases with age and generally varies from 5 to 15% in women over 35 years of age [7]. The prevalence of HPV infection also remains high in elderly women; in certain populations [8]. There are several methods for identifying HPV on cervical lesions. [2]. It is particularly the HPV testing carried out with a commercial kit (Digene Cervical Sampler) including a cervical brush, the "Hybrid" test. Capture II "(HCII) (Digene, Gaithersburg, MD, USA) which is used to study high-risk HPV infection [9], and amplification of a short region of viral DNA by the technique of polymerase chain reaction (PCR). In Democratic Republic of Congo and more specifically in Lubumbashi for lack of adequate equipment, none of these methods are used. The only possibility of detecting HPV infection on cervical smears in this country is to proceed indirectly through microscopic research of koilocytosis (stigmas of papillomavirus replication). This study aims to show that the koilocytosis on the cervical smear is the only possibility for the detection of HPV in intra-epithelial neoplasia in Lubumbashi and also it aims to determine the frequency of HPV carriage in the population of Lubumbashi and analyze the relationship between HPV infection and cervical intra-epithelial neoplasia.

2. Method

2.1. Type of Study

This is a cross-sectional analytical study based on an analysis of cervical smear for the campaign of intra-epithelial neoplasia detection organized in March 2012 by the Congolese League against Cancer registry in Lubumbashi.

2.2. Selection of Cases

Before carrying out this study, we collected slides of cervical smear from 629 sexually active women living in Lubumbashi who voluntarily participated in the precancerous cervical lesions screening campaign organized in March 2012 by the Congolese League Against the cancer.

2.3. Inclusion Criteria

Women who participated in the campaign with an inflammatory smear or intra-epithelial neoplasia of the cervix according to the modified Bethesda classification were included in this study, resulting in a total of 545 cases.

2.4. Exclusion Criteria

Women whose smears were inadequate or within normal limits were excluded from this study, for a total of 84 women.

2.5. Cytology Treatment

The cervical smears were stained according to the conventional Harris-Shorr technique by following the

following steps: Fixation in methanol for 15 minutes, passage in three diluted ethyl alcohol baths at 95°, 70°, and 50°, rinsing with distilled water, staining with Harris hematoxylin for 5 minutes, rinsing with distilled water, passing through the bath of ammonia alcohol for 15 seconds, rinsing with water distilled, the successive passage in three baths of ethical alcohol diluted at 50°, 70° and 95°, the staining with Shorr for three minutes, the passage in two baths of alcohol of 95° and 100°, the passage in two xylene baths and finally the break of covers object before microscopic analysis [10].

2.6. Cytopathological Analysis

The cytopathological analysis was performed at the Pathological Department of the University Hospital of Lubumbashi independently by three experienced pathologists using the modified Bethesda classification [11]. In the event of a discrepant diagnosis between the three pathologists, a consensus should be found based on the documentation. Six classes were retained: (1) inadequate smear, (2) smear within normal limits, (3) cytology compatible with inflammatory changes (4) and epithelial neoplasia. This category had 2 sub-classes: low grade and high grade intraepithelial neoplasia. The search for koilocytosis (cells carrying a perinuclear halo) [12] was systematically made to the X40 objective on all cases of smear with inflammatory alterations and those with intra-epithelial neoplasia of the cervix.

2.7. Parameters

There is the age of women examined and the morphological aspects of cervical smears as well as the presence of koilocytosis.

2.8. Statistical Analyzes

Statistical analyzes were done on a Compaq CQ58 laptop. The Epi Info 2011 software was used for data processing. Proportions were calculated and expressed as a percentage. The exact Fisher test, the Chi-square test, the Odds ratio and the confidence interval were used for the comparison of proportions. The value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Age of Women

The average age of the 545 women in Lubumbashi selected in this study was 40 ± 5.4 years. The age limits ranged from 22 to 65 years old.

3.2. Cytological Classification

Intra-epithelial neoplasia of the cervix constituted 15% of cases ($n = 82$), inflammatory lesions accounted for 85% of cases ($n = 463$). Table 1 illustrates these results.

Table 1. Cytological evaluation of smears (n=545).

Evaluation	Number	%
CIN	82	15
Inflammatory smears	463	85
Total	545	100

CIN (Cervical Intra-epithelial neoplasia).

3.3. Association Between HPV and Intra-Epithelial Neoplasia

Women with HPV stigma (koilocytosis) were 38.3 times more likely to develop intra-epithelial neoplasia compared to those who did not. This difference was statistically significant (OR = 38.3, 95% CI = 16.3-90.3, $\chi^2 = 137.9$, $p < 0.0001$). Table 2 reports these observations.

Table 2. Koilocytosis and cervical lesions (n=545).

Koilocytosis	CI Nnumber (%)	Inflammatory smears Number (%)	Total
Absent	6 (7.3%)	348 (75.2%)	354
Present	76 (92.7%)	115 (24.8%)	191
Total	82	463	545

OR=38,3; IC 95%=16,3-90,3; $\chi^2=137,9$; $p<0,001$.

3.4. Koilocytosis and Patient Ages

The distribution of women with koilocytosis shows that 55% of women (n = 105) with koilocytosis were older to 35 years while the remaining 45% (n = 86) were aged at under 35 (Table 3).

Table 3. Age of women and koilocytosis (n=191).

Age	Fréquency of koilocytosis	%
≤ 35 years	86	45
≥ 36 years	105	55
Total	191	100

3.5. Correlation Between Koilocytosis and CIN Grade

Koilocytosis were found in 91.9% (n = 57) of low-grade precancerous lesions and 95% (n = 19) of precancerous high-grade lesions, respectively. This difference was not statistically significant (OR = 1.7, 95% CI = 0.2-15.2, exact Fisher, $p = 1$) (Table 4).

Table 4. Correlation between CIN grade and koilocytosis (n=82).

Koilocytosis	High grade CI Nnumber and (%)	Low grade CI Nnumber and (%)
Absent	1 (5%)	5 (8,1)
Présent	19 (95)	57 (91,9)
Total	20	62

OR=1, 7; IC 95%=0,2-15,2; Fisher exact; $p=1$.

4. Discussion

4.1. HPV Detection Methods

There are several methods for detecting and identifying HPV in cell harvesting. The most sensitive is based on the amplification of a short region of viral DNA by the

polymerase chain reaction (PCR) technique and the use of degenerate primers or consensus primers, localized in the L1 gene, which amplifies a broad spectrum of HPV genotypes with genital tropism [2]. This technique is widely used in the detection and identification of HPV infections [13-15]. Other tests are based on the detection of viral DNA by molecular hybridization, without prior amplification; their sensitivities are lower than those based on PCR or the hybrid capture technique [2]. The Hybrid test is also used [16] and can detect a potentially oncogenic HPV (or a non-oncogenic HPV), but without identifying the genotype involved [9]. For lack of adequate equipment, all these techniques are not used in Lubumbashi, the only possibility to detect HPV indirectly in this city is to identify the koilocytosis in cervical smears. This cytologic abnormality is frequently associated with intra-epithelial neoplasia, and represents the stigma of papillomavirus replication in the [6].

4.2. Age of Women

The mean age of women in the present study is 40 ± 5.4 years and the range is 22 to 65 years. Boumba et al., found an average age of 43.6 ± 9.5 years (range 16 to 72 years) in a population of Brazzaville [17]. These observations show that these two populations live in the same socio-demographic conditions.

4.3. Frequency of HPV Infection in the Population

The frequency of HPV infection in Lubumbashi is 35% in this study. Boulanger et al., found a frequency of HPV carriage of 14.32% in the French population [16]. Boumba et al. found a frequency of 23.5% HPV in the Congolese population (Brazzaville) [1]. The sensibility and the specificity of the techniques used for the identification of HPV explain these differences of frequency.

4.4. HPV and Intra-Epithelial Neoplasia of the Cervix

The stigmata of HPV were found in 92.7% of cases of intra-epithelial neoplasia in this study. Boumba et al. had observed HPV infection in 89.6% of cases of intra-epithelial neoplasia [17]. Many studies demonstrate a high frequency of human papillomavirus (HPV) infection in intra-epithelial neoplasia [18].

4.5. HPV and Grade of Cervical Intra-Epithelial Neoplasia

HPV stigmata were found in 91.9% of cases of low-grade intraepithelial neoplasia and in 95% of those of high grade in this study, and this difference was not statistically significant. Boumba et al., showed that HPV infection was 80.9% low-grade neoplasia and all high-grade neoplasms were HPV-positive [17]. These observations show that HPV infection was predominantly found in both low-grade and high-grade neoplasia.

4.6. HPV Risk Factor for Intra-Epithelial Neoplasia

This study shows that women with HPV stigmas were 38.3

times more likely to develop cervical intra-epithelial neoplasia compared to those without HPV stigma. Kousky *et al.*, reported that the risk of cervical intra-epithelial neoplasia was 11 times more in women with HPV infection than in women without HPV [19].

4.7. HPV Stigmas and Patient Age

The stigma of HPV is found in 55% of women aged 36 years and over and 45% of those aged 35 and under in this study. According to observations made by Boumba *et al.*, although they noted a 32% incidence of HPV infection in women under 30, the difference in HPV frequency by age group was not statistically significant [1]. Young women are more affected by HPV infection, older women are also infected with this virus [21].

5. Conclusion

The stigmas of HPV are found in a not insignificant proportion in the women of Lubumbashi having sexual activity regardless of their age. These stigmas are strongly associated with intra-epithelial neoplasia of any grade. It is therefore important to put in place the most sensitive means of detecting HPV in order to contribute to the fight against cancer of the cervix in Lubumbashi.

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