



Cervical Neoplasia: Papillomavirus Infection and Resistance to Anti Tumor Immunity

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To cite this article:

Mwenzé Didier, Kyabu Véronique, Bokambandja Fabrice, Kasamba Eric, Mukalay Abdon, Kalenga Prosper, Lebwaize Bienvenu. Cervical Neoplasia: Papillomavirus Infection and Resistance to Anti Tumor Immunity. *International Journal of Clinical Oncology and Cancer Research*. Vol. 5, No. 4, 2020, pp. 82-92. doi: 10.11648/j.ijccocr.20200504.12

Received: October 15, 2020; **Accepted:** October 28, 2020; **Published:** November 23, 2020

Abstract: This study aim to identify the expression of resistance to antitumor immunity on cellular and tissue alterations predictive of Human Papillomavirus infection as well as to establish the relationship between this resistance and the grades of Intraepithelial neoplasms of the cervix in patients from Lubumbashi and Kinshasa in the Democratic Republic of Congo. This is a study on cervical biopsies whose diagnosis of intraepithelial neoplasia was confirmed by a routine histopathological examination in two pathology laboratories in the Democratic Republic of Congo, or the laboratory of Lubumbashi and the LEBOMA laboratory of Kinshasa. The paraffin blocks were selected during a period from March 2017 to March 2020 at the laboratory of Lubumbashi and at the LEBOMA laboratory in Kinshasa. Laboratory manipulations were carried out at the molecular pathology unit of the LEBOMA Pathological Anatomy and Cytology Cabinet in Kinshasa. Papillomavirus infection was retained by the presence of predictive cellular and tissue alterations. The expression of PD-L1 signifies resistance to anti-tumor immunity. The following results were observed; A total of 50 cases of intraepithelial neoplasia were recorded in a set of 107 patients with cervical lesions, i.e. a frequency of 46.7%. The cytological and histological alterations predictive of papillomavirus infection were observed in the following proportions: For cytological changes; koilocytosis was present in 92%, bi-nucleation was observed in 24% and dyskeratosis was identified in 46%, ie 46, 12 and 23 cases, respectively, out of 50 cases of cervical neoplasia recorded. The proportions of histological alterations are presented as follows: 62% of papillomatosis, 86% of acanthosis, 54% of basal hyperplasia and 34% of intraepithelial capillaries, i.e. 31, 43, 27 and 17 cases out of respectively. The 50 cases of intraepithelial neoplasia of the cervix recorded. PD-L1 expression was observed in 16 cases out of all intraepithelial neoplasia, ie 32% of cases. PD-L1 is mainly expressed in high-grade intraepithelial neoplasias, i.e. 81.25% 95% CI=54.35-95.95 and those involving bi-nucleation (OR=26.66 95% CI=4, 63-153.57, Fisher exact, p<0.01), the intraepithelial capillaries or 37.5%; 95% CI=15.20-64.57. It emerges from this study the expression of PD-L1 is not uniform over all the cyto-histological alterations predictive of human papillomavirus infection during neoplasias. intraepithelial of the cervix, but it is much more expressed in high-grade intraepithelial neoplasias and in those comprising the predictive alterations of Papillomavirus infection like the bi-nucleation and the intraepithelial capillary.

Keywords: Intra-Epithelial Neoplasia, HPV, PD-L1

1. Introduction

Intraepithelial neoplasia of the cervix is associated with the persistence of Papillomavirus infection within epithelial cells of the cervix [1–3]. This persistence of Papillomavirus infection is due to several mechanisms [4–7] and causes morphological changes in the cells of the cervix [8]. In several published studies, Papillomavirus infection in the intraepithelial neoplasia of the cervix is revealed by indirect molecular biomarkers [9–12]. Identifying certain indirect biomarkers of Papillomavirus infection is part of routine practice in the diagnosis and prognosis of intraepithelial neoplasia of the cervix [13–17]. The most desired indirect biomarker of Papillomavirus infection in cervical intraepithelial neoplasia is P16 INKa; Its overexpression has been shown in several studies to be as associated with intraepithelial neoplasia of the cervix secondary to high carcinological Papillomavirus infection [18–20]. Several other authors show that the biomarkers Ki67 and TP53 are also considered to be a marker of high oncogenic Papillomavirus infection in intraepithelial neoplasia of the cervix [21–23]. Further research is focusing on identification of viral oncoproteins E6 and E7 as markers of high-risk Papillomavirus infection in intraepithelial neoplasia [24–26]. PD-L1 is involved in the molecular mechanism of cervical carcinogenesis by resistance to anti-tumor immunity of intraepithelial neoplasia cells [27, 28]. Most of the published studies on PD-L1 concern its identification in invasive or metastatic cancers [29–31]; But there are very few published studies on PD-L1 in intraepithelial neoplasia of the cervix. These studies evaluated the expression of PD-L1 in intraepithelial neoplasia of the cervix [32–35]. In addition to molecular biomarkers, persistent Papillomavirus infection at the cell level is characterized by morphological stigmas [8]. Several morphological stigmas of Papillomavirus infection are identified namely koilocytosis, acanthosis, binucleation, intraepithelial capillaries, dyskeratosis and papillomatosis and hyperplasia of the basal layer. Numerous published studies show that papillomatosis is a common morphological marker of papillomavirus infection [36, 37]. Koilocytosis is considered to be the pathognomonic morphological marker of papillomavirus infection [38–40]. Several studies demonstrate the existence of relationships between some molecular biomarkers and certain morphological markers of Papillomavirus infection [39–42]. Most of these studies focus on the relationship between P16, Ki 67 and P 53 with koilocytosis [43, 44].

To date, according to the literature available to us, only one study on the immunohistochemical biomarkers of intraepithelial neoplasia of the cervix has been carried out in the Democratic Republic of the Congo and more precisely in Kinshasa. This study shows the value of P16 and KI 67 in the diagnosis of precancerous lesions [45]. No study published in the Democratic Republic of Congo in general and in Lubumbashi in particular on the relationship that would exist between the morphological stigmas of Papillomavirus infection and the expression of the molecular biomarker (PD-L1) of resistance to anti- tumor immunity in intra-epithelial

neoplasia of the cervix. The objective of this study is to identify the presence of resistance to anti-tumor immunity in the cellular and tissue alterations induced by human papillomavirus infection and to establish the relationship that would exist between this resistant and the grade of intraepithelial neoplasia of the cervix.

2. Method

2.1. Type of Study

This is a cross-sectional analytical study based on the identification of cyto-histological alterations predictive of Papillomavirus infection and expression of PD-L1 on cervical biopsies diagnosed with intraepithelial neoplasia recorded during this study.

2.2. Case Selection

The patients were selected from all patients with cervical lesions recorded at the Pathological Anatomy and Cytology laboratory of University Clinics in Lubumbashi and at the LEBOMA Cabinet in Kinshasa. The selection was made by an exhaustive sampling taking into account all the cases of biopsy of the uterine cervix diagnosed with intraepithelial neoplasia of the cervix recorded during the period of our study, i.e. 50 cases out of a total of 107 patients. carriers of cervical lesions. We then selected all the paraffin blocks from these patients for laboratory manipulations.

2.3. Inclusion Criteria

The patients diagnosed with intraepithelial neoplasia of the uterine cervix whatever the grade according to the histological classification criterion and whose paraffin blocks found allowed recutting were included in this study, i.e. a total of 50 cases.

2.4. Exclusion Criteria

Patients whose cervical biopsies reveal an invasive carcinoma or a tumor of the columnar or glandular epithelium or cervicitis were excluded from this study.

2.5. Hematoxylin – Eosin Staining.

Paraffin sections of approximately 4 microns were stained at the LEBOMA Pathological Anatomy and Cytology laboratory in Kinshasa by the manual technique of routine histological staining, going through the following steps:

- Dewaxing in the xylene bath.
- Nuclear staining with hematoxylin.
- Extranuclear staining with eosin.
- Xylene lightening.
- The assembly of blades.

2.6. PD-L1 Immunostaining

PD-L1 immunostaining was performed at the LEBOMA Pathological Anatomy and Cytology Laboratory in Kinshasa

using the manual immunohistochemistry technique through the following steps:

- Making sections of paraffin 4 microns thick.
- Deparaffinization and rehydration of slides.
- Pretreatment unmasking the antigens.
- Antigenic labeling (primary and secondary antibodies).
- Counterstaining with hematoxylin.
- Fixing a cover slip for conservation.

2.7. Identification of Predictive Alterations of Human Papillomavirus Infection

Cyto-histological changes predictive of human papillomavirus infection were looked for under an optical microscope on the slides stained with eosin hematoxylin. Koilocytosis has been identified by the presence of a clear perinuclear halo [46]. Otherwise koilocytosis is absent. The presence of bi-nucleation is found when the epithelial cells carry two nuclei. Dyskeratosis has been looked for under an optical microscope by identifying different keratinization disorders, namely para keratosis or hyperkeratosis. Papillomatosis is retained when there is a finger-like appearance of the hyperplastic Squamous Epithelium. Acanthosis is defined by an abnormal multiple stratification of the Malpighian layer of the lining epithelium of the ectocervix. Basal layer hyperplasia is characterized by nuclear hyperchromatism in the cells of the basal layer accompanied by multiple stratification of this layer. Intraepithelial capillaries are formed by abnormal intraepithelial vascular formations.

2.8. Grade of Intraepithelial Neoplasia

The histological grade was established by 3 pathologists from university clinics in Lubumbashi, following the classification criteria for intraepithelial neoplasia proposed by WHO in 2003. All 3 grades were retained; or grade 1 when the tissue alterations affect only less than the lower third of the epithelial layer; grade 2 when the histological changes are in more than half of the epithelial layer; grade 3 if the tissue alterations involve cells in almost all epithelial layers [47, 48].

2.9. The Expression of PD-L1

PD-L1 expression was qualitatively assessed by reading under a light microscope of slides incubated with the primary PD-L1 antibody [49]. Two results were obtained, either positivity (presence of cytoplasmic and membrane labeling of epithelial cells) or negativity (total absence of membrane or cytoplasmic labeling of epithelial cells).

2.10. Study Parameters and Statistical Analyzes

The following parameters were examined in this study: the morphological parameter of Papillomavirus infection (Koilocytosis, bi-nucleation, Dyskeratosis, Acanthosis, Papillomatosis, Intraepithelial capillaries and Basal layer hyperplasia), the grade of neoplasia and the molecular parameter of resistance to anti-tumor immunity (expression of PD-L1).

Statistical analyzes were done on a Compaq CQ58 laptop. Epi Info 2011 software which was used for data encoding and processing. The proportions were expressed as a percentage. The 95% confidence interval, odds ratio, and Fisher's exact test were used to compare the results.

3. Results

3.1. Frequency of Intraepithelial Neoplasia of the Cervix in Patients with a Cervical Lesion

The frequency of intraepithelial neoplasms recorded in this study was 50 cases out of 107 cervical biopsies, or 46.7%. Table 1 illustrates these observations.

Table 1. Frequency of intraepithelial neoplasia of the cervix in patients with cervical lesions (n=107).

	Number	%
Intra-epithelial Neoplasia	50	46,7
Other cervicals lesions	57	53,3
Total	107	100

3.2. Distribution of Intraepithelial Neoplasia of the Cervix According to PD-L1 Expression

PD-L1 expression in intraepithelial neoplasia was identified in 16 cases or 32% of cases with a 95% confidence interval. (Table 2)

Table 2. Frequency of PD-L1 expression in cervical intraepithelial neoplasms. (n=50).

PD-L1 Expression	Number	%
Positive	16	32,00%
Négative	34	68,00%
Total	50	100,00%

3.3. Distribution of Cytological Alterations Predictive of Papillomavirus Infection in Intraepithelial Neoplasms of the Cervix

The various cytological alterations predictive of papillomavirus infection have been observed in the following proportions in intraepithelial neoplasms of the cervix; koilocytosis was identified in 46 cases or 92% of cases with an interval. The presence of binucleated cells was detected in 12 cases or 24% of cases. The presence of dyskeratotic cells within intraepithelial neoplasms was identified in 23 cases, ie 46%. (Table 3)

Table 3. Frequency of cytological alterations predictive of papillomavirus infection in intraepithelial neoplasms of the cervix (n=50).

Cytological alterations	Presence Number (%)	Absence Number (%)
Koilocytosis	46 (92)	4 (8)
Bi nucleation	12 (24)	38 (76)
Dyskeratosis	23 (46)	27 (54)

3.4. Association Between the Presence of Koilocytosis and PD-L1 Expression

In cervical intraepithelial neoplasms with koilocytosis,

PD-L1 was expressed in 28.3% (n=13 of 46); In those without koilocytosis, PD-L1 was expressed in 75.0% (n=3 of 4) of PD-L1, but this difference was not statistically significant (OR=0.13, 95% CI=0, 01-1.38, Fisher exact, p=0.09) (Table 4).

Table 4. Koilocytosis and PD-L1 in intraepithelial neoplasms of the cervix (n=50).

Koilocytosis	PD-L1 Positive	PD-L1 Negative	Total
Presente	13 (28,3%)	33 (71,7%)	46 (100%)
Absente	3 (75,0)	1 (25,0%)	4 (100%)
Total	16	34	50

OR=0,13, IC 95%=0,01-1,38, exact Fisher, p=0,09.

3.5. Association Between Bi Nucleation and PD-L1 Expression

In cases of intraepithelial neoplasia of the cervix containing the binuclear cells, PD-L1 was expressed in 83.3% (n=10 of 12); In those without bi-nucleation, PD-L1 was expressed in 15.8% (n=6 of 38) of PD-L1. This difference was statistically significant (OR=26.66 95% CI=4.63-153, 57, Fisher exact, p <0.01) (Table 5).

Table 5. Bi-nucleation and PD-L1 in intraepithelial neoplasias of the cervix (n=50).

Bi nucleation	PD-L1 Positive	PD-L1 negative	Total
Presente	10 (83,3%)	2 (16,7%)	12
Absente	6 (15,8%)	32 (84,2%)	38
Total	16	34	50

OR=26,66 IC 95%=4,63-153,57, exact Fisher, p <0,01.

3.6. Association Between Dyskeratosis and PD-L1 Expression

In cases of intraepithelial neoplasia of the cervix with dyskeratotic cells, PD-L1 was expressed in 17.4% (n=4 of 23); In those without dyskeratosis, PD-L1 was expressed in 44.4% (n=12 out of 27) of PD-L1, but this difference was not

statistically significant (OR=0.26 95% CI=0, 07-0.9, Fisher exact, p=0.06) (Table 6).

Table 6. Dyskeratosis and PD-L1 in intraepithelial neoplasms of the cervix (n=50).

Dyskeratosis	PD-L1 Positive	PD-L1 negative	Total
Presente	4 (17,4%)	19 (82,6%)	23
Absente	12 (44,4%)	15 (55,6%)	27
Total	16	34	50

OR=0, 26 IC 95%=0,07-0,9, exact Fisher, p=0,06.

3.7. Distribution of Tissue Alterations Predictive of Papillomavirus Infection in Intraepithelial Neoplasms of the Cervix

The distribution of tissue alterations predictive of papillomavirus infection within intraepithelial neoplasms of the cervix shows the presence of papillomatosis, acanthosis, hyperplasia of the basal layer and intraepithelial capillaries in proportions variable, i.e. 62, 86, 54 and 34% of cases, respectively. (Table 7).

Table 7. Distribution of tissue alterations predictive of papillomavirus infection in intraepithelial neoplasms of the cervix (n=50).

Tissue alterations of HPV infection	Presence Number (%)	Absence Number (%)
Papillomatosis	31 (62)	19 (38)
Acanthosis	43 (86)	7 (14)
Hyperplasia of the basal layer	27 (54)	23 (46)
Intra-epithelial capillaries	17 (34)	33 (66)

3.8. PD-L1 Expression According to Tissue Lesions Predictive of Papillomavirus Infection

Intraepithelial neoplasias comprising intraepithelial capillaries constitute 37.5% of those expressing PD-L1 and those comprising basal hyperplasia represent 25%; those with papillomatosis and acanthosis account for 18.75% of cases of PD-L1 expression. These differences were statistically significant at the 95% confidence interval. (Table 8)

Table 8. Distribution of PD-L1 expression according to tissue alterations predictive of Papillomavirus infection.

Alterations	PD-L1 Positive	%	IC 95%
Papillomatosis	3	18,75	4,05-45,65
Acanthosis	3	18,75	4,05-45,65
Intra-epithelial capillaries	6	37,5	15,20-64,57
Hyperplasia of the basal layer	4	25	7,27-52,83
Total	16	100	

3.9. PD-L1 Expression According to the Grade of Intraepithelial Neoplasia of the Cervix

In all intraepithelial neoplasias of the cervix expressing PD-L1, grade 3 neoplasias represent 81.25% of cases and those of grade 2 and 1 represent 12.50 and 6.25% of cases, respectively.. This difference was statically significant at the 95% confidence interval (Table 9).

Table 9. Distribution of PD-L1 expression according to the grade of intraepithelial neoplasia of the cervix (n=16).

Grade of CIN	PD-L1 Positive	%	IC 95%
CINI	1	6,25	0,16-30,23
CIN II	2	12,50	1,55-38,35
CIN III	13	81,25	54,35-95,95
Total	16	100	

CIN (Cervical intra-epithelial neoplasia).

4. Discussion

4.1. Frequency of Intraepithelial Neoplasia

This study shows that out of a total of 107 women with a lesion of the cervix, 50 of them have intraepithelial neoplasms of the cervix, a frequency of 46.7%. In the study of intraepithelial neoplasia of the cervix in Brazilian women, the authors found 90 cases of intraepithelial neoplasias in 150 cervical samples, or 60% of cases [50]. Mwenze et al, in a study carried out in Lubumbashi on screening for cervical cancer in the population, observed a frequency of intraepithelial neoplasia of the cervix equal to 13% within the population of Lubumbashi [51]. During the study on the frequency of cervical intraepithelial neoplasia in users of contraceptive pills, the authors found a frequency of 14% of cases [52]. In the cervical cancer screening campaign carried out in the east of the Democratic Republic of Congo, the frequency of intraepithelial neoplasms of the cervix was 7.45% in the screening population [53]. In the study of screening for cervical intraepithelial neoplasia in an urban population in Nigeria, intraepithelial lesions accounted for 7.7% of all participants [54]. In the study on the prevalence of cervical intraepithelial neoplasia in patients attending the Maternity Department of the University Hospital of Minia, the authors also found a prevalence of intraepithelial neoplasia equal to 7.7% in the study population [55]. The prevalence of cervical intraepithelial neoplasias in the female population of Zaria in northern Nigeria was 4.8% [56]. In the Brazilian study on the incidence of human cervical papillomavirus infection and cervical intraepithelial neoplasia in women with HIV positive or negative status, the incidence of intraepithelial neoplasia was 8.8% and 4.6% respectively in HIV positive and HIV negative women [57]. The study investigating the prevalence and genotypic distribution of human papillomavirus in invasive cancer and intraepithelial neoplasias of the cervix as well as in asymptomatic women in Southeast China, the prevalence of intraepithelial neoplasias of the cervix uterus was 9.02% [58]. Although the frequency of intraepithelial neoplasia of the cervix appears to be low in the population, it is generally higher in communities characterized by a high rate of HIV infection in the female population [56]. However, if these HIV positive patients are on antiviral therapy, the prevalence of intraepithelial cervical neoplasia is low [59]. Although intraepithelial neoplasia is the most common lesions of the cervix [60]. Their proportions are much greater when the studies concern patients with clinically identifiable lesions [50]. But in cases where studies are done to screen a population for cervical cancer, the frequencies of intraepithelial neoplasms are relatively low [21].

4.2. Cytological Alterations Predictive of Papillomavirus Infection

Koilocytosis, bi-nucleation and dyskeratosis were observed in intraepithelial neoplasias of the cervix at relatively the

following frequency of 92.24 and 46% in this study. During the study on the quantitative and qualitative detection of Papillomavirus in precancerous lesions of the cervix, the authors observed koilocytosis in a frequency of 61.2% of intraepithelial neoplasms of the cervix [61]. In a study looking for papillomavirus infection in women from Lubumbashi, the authors found the presence of koilocytosis in 92.7% of cases of intraepithelial neoplasia. In the post-coital hemorrhage and cervical dysplasia study, the proportion of koilocytosis was 15.6% (64/411) in CIN1 and 0.7% for high-grade pathology of CIN 2 or more (3/411) [62]. Koilocytosis is considered to be a common cytopathic effect in patients infected with human papillomavirus, this change is caused by any genotypes of human papillomavirus regardless of its oncogenic potential [63]. During the retrospective analysis of the clinical significance of CK7, HPV-L1, and koilocytosis in patients with cervical intraepithelial lesions, koilocytosis was detected in 52.0% of low-grade intraepithelial lesions and in 64.3% in high grade ones [64]. During the study comparing different diagnostic methods of human papillomavirus infection in Egyptian women with lesions of the cervix; koilocytosis has been identified in 62.50% of grade I cervical intraepithelial neoplasms [65]. Some authors have used morphological consensus criteria based on the presence of koilocytosis for the diagnosis of intraepithelial neoplasia of the cervix. These authors observe that: koilocytosis is absent in 55.5% of CIN1 and 5.5% CIN2 and in no case classified as CIN3 [66]. Analyzing the histological criteria for the diagnosis of human papillomavirus infection, the authors find that koilocytosis is observed in 34% of patients with grade 1 cervical intraepithelial neoplasia [67]. In the prognostic and reproducibility study of koilocytosis in cervical intraepithelial neoplasms, koilocytosis was found in 62% of CIN 1s and 70% of CIN 2s [68]. During the study of cytopathological alterations of human papillomavirus infection and the severity of cervical intraepithelial neoplasms, the authors noted that koilocytosis was found in 63% of women with a histopathological diagnosis of CIN 1. This sign was observed in 26.2% and 25.7% of women with a diagnosis of CIN 2 and CIN 3, respectively [69]. In the study of koilocytosis, X-chromatin and HSV-2 in cervical smears in Nepal, koilocytosis was observed in 42.1% of cases of CIN I [70]. In the study of the histopathological aspects of cancerous and cancerous lesions of the cervix performed at the Department of Pathology of the College of Medicine of India, koilocytosis was observed in 8 cases (22.2%) of intraepithelial neoplasia of the cervix, including 4 cases each of CIN 1 and CIN 2 [71]. These large proportions of koilocytosis observed are explained by the fact that koilocytosis is a common morphological feature of high-risk and low-risk oncogenic papillomavirus infection in intraepithelial neoplasms of the cervix [72]. In the study carried out in Brazil and relating to the comparison of classical and secondary cytological criteria relating to hybrid capture for the diagnosis of cervico-vaginal infection by the human papillomavirus, bi or multi nucleation was identified in (68 cases) and dyskeratosis (36 cases) with no statically significant difference [73]. By examining the importance of binuclear

cells in cervical specimens using the FISH technique; Binucleated cells were identified by the presence of nuclei pressed against each other as positive compression, and their relationship to the relative light units (RLU) of hybrid DNA capture 2 (HC2) was determined; the authors found that binuclear cells (positive compression) were present in 95.2% of cases of intraepithelial neoplasia of the cervix and that their number increased with the grades of intraepithelial neoplasia of the cervix [74]. During cervical cancer screening performed at Kale Hospital in the Pathology Department of the University of Tokyo's Faculty of Medicine in Japan, microscopic images of precancerous lesions were analyzed on a computer at the 'using a digital image analysis program; the number of dyskeratotic cells observed was 813 out of a total of 4049 cells analyzed, i.e. 20.07% [75]. During the epidemiological and diagnostic study of precancerous cervical lesions carried out in the region of Sidi Bel Abbes (north-western Algeria), dyskeratosis was identified in 8% of high-grade intraepithelial neoplasms [76]. Evaluation of dyskeratosis on cervicovaginal swabs in Turkish women shows that it is much more associated with a histological diagnosis of high grade intraepithelial neoplasia ($p=0.8$) [77]. In a study investigating factors associated with cervico-uterine changes in women from the city of Saint Catherine in western Brazil, dyskeratotic and binucleate cells were identified in 11.8% of intraepithelial neoplasias of the cervix. [78]. A study carried out at the Tertiary Hospital of India on aspects of cervical cytology using Papanicolaou stain showed that binuclear cells are found in both low-grade and high-grade intraepithelial neoplasias [79]. In a study conducted at the pathology laboratory at Slatina Hospital on the cytological identification of pre-cancerous cervical lesions in asymptomatic female populations, dyskeratotic cells were only identified in low-grade intraepithelial lesions [80]. Analysis of cervical intraepithelial lesions carried out in a study conducted at the Department of Pathology of the General Hospital of Women's Health of the College of Medicine of Kwandong University in Korea, the authors found the bi-nucleation at breast intraepithelial neoplasia of the cervix in 71 and 18.4% respectively for low and high grade lesions [81]. Analyzing cervical swabs taken after colposcopy from women in the Calgary health region in Canada, the authors noted that dyskeratosis was found in 10% of low-grade intraepithelial neoplasms [82]. These observations show that koilocyte cells, binucleate and dyskeratosis are identified in intraepithelial neoplasms in different proportions. These differences in proportions may be due to the variation in human papillomavirus genotypes in the different study settings and to the difference in techniques used to identify these cells. In fact, certain human papillomavirus genotypes are much more associated with cellular alterations of the bi-nucleation and dyskeratosis types. Quad to methods of identifying cells within cervical intraepithelial neoplasms, although histopathology is the gold standard of diagnosis, this method has poor reproducibility when using different observers, this situation arouses subjectivity; hence the need to couple histopathology with modern diagnostic techniques such as molecular biology and immunohistochemistry.

4.3. PD-L1 Expression

The frequency of PD-L1 expression in cervical intraepithelial neoplasms in this study was 32% in this study. In the study investigating increased expression of PD -L1 in intraepithelial neoplasms and cervical cancers, the authors found PD-L1 expression in 95% of intraepithelial neoplasias [33]. In the study of factors of recurrence of intraepithelial neoplasia after cervical conization, PD-L1 expression was observed in 60% of recurrent neoplasias [83]. The study analyzing the expression of PD as an immune checkpoint in cervical and vulvar intraepithelial neoplasias, PDL1 expression was observed in 10% of intraepithelial neoplasia [84]. Since PD-L1 is expressed that on cells infected with high-risk oncogenic papillomavirus [85], these differences in the frequency of expression translate that the distribution of high-risk oncogenic papillomavirus is not identical in different populations. PD-L1 is a high-risk oncogenic papillomavirus biomarker [86].

4.4. Cytological Changes Predictive of Papillomavirus Infection and Expression of PD-L1

PD-L1a expression was variable in the different cytological alterations predictive of human papillomavirus infection in this study, i.e. 28.3% in koilocytosis, 83.3% within bi-nucleation and 17.4%. In dyskeratosis. Indeed, the low expression of PD-L1 in koilocytic cells is explained by the fact that PD-L1 is a molecular biomarker much more specific for papillomavirus infection with high oncogenic risk and resistant to anti-tumor immunity. Its overexpression in intraepithelial neoplasias of the cervix is induced by the E7 oncoprotein of papillomavirus type 16 [85]. While koilocytosis is a much more general stigma of high and low risk papillomavirus infections of oncogenicity [72]. Binuclear cells strongly express PD-L1 and other biomarkers of cell proliferation and transformation because these cells are formed from a fusion of several cells in order to resist the body's defense system [87]. The authors reveal that Ki-67 is highly expressed in binucleated cells (more than 50%) [87]. In the study examining the importance of the pathologic anatomy of precancerous lesions and cervical cancer in clinical practice, the authors state that binuclear cells are generally labeled with P53 [88]. Binucleated cells in intraepithelial neoplasia of the cervix show overexpression of the cell cycle bound p16 marker [89]. By analyzing the atypical epithelial cells induced by the different genotypes of human papillomavirus, the authors state that the binucleated cells are characterized by an expression of P16 [90]. While performing immunocytochemistry on the exfoliative cytology of several organs, including those of the female genital tract, the authors find that binuclear cells are labeled with PD-L1 [91]. Several studies show evidence of PD-L1 expression on binucleated and dyskeratotic cells in many cancers induced by the papillomavirus. It is particularly during the study of head and neck cancer secondary to papillomavirus infection where parabasal layer binuclear cells and middle layer dyskeratosis strongly express PD-L1

[92]. Searching for a viral origin in cases of squamous cell carcinoma of the mouth, the authors identified dyskeratotic cells expressing PD-L1 [93]. In precancerous lesions of the esophagus induced by papillomavirus infection, dyskeratotic cells expressing the PD-L1 [94]. These observations show that several molecular biomarkers including PD-L1 are expressed on binucleate and dyskeratotic cells induced by human papillomavirus infection.

4.5. Tissue Alterations Predictive of Papillomavirus Infection

The tissue alterations induced by the papillomaviruses identified in this study are in different proportions: 86% for acanthosis, 62% for papillomatosis, 54% for basal hyperplasia and 34% for intraepithelial capillaries. The most identified tissue alterations predictive of papillomavirus infection in intraepithelial neoplasms of the cervix or other organs are acanthosis and papillomatosis. In the study on the clinical-pathological features of high-grade intraepithelial lesions of the cervix conducted at the Department of Pathology at Severance University Hospital in Korea, the authors identified acanthosis and papillomatosis as tissue alterations induced by papillomaviruses in intraepithelial neoplasms of the cervix [62]. By analyzing the histological lesions of human papillomavirus infection in precancerous and cancerous lesions of the cervix in Tunisian women, the authors had also identified papillomatosis and acanthosis in addition to cytological alterations [63]. In the study of tissue expression of VEGF in intraepithelial neoplasias and cervical cancer, the authors noted the presence of intraepithelial capillaries in 33.33% of intraepithelial neoplasias [64]. During the study on the expression of plasminogen activator type MMP-2, MMP-9 and urokinase in cervical intraepithelial neoplasias, the presence of intraepithelial capillaries was revealed by the expression of VEGF and was 10.2% and 58.6%, respectively, in low- and high-grade intraepithelial neoplasms [65]. In the study evaluating papillomavirus infection and the effects of smoking on cell proliferation in the epithelial layers of cervical neoplasms, the proportion of basal layer hyperplasia was identified by the expression of the E6 and it varied according to the grade. It was much higher in grade 3 lesions compared to normal epithelial tissue (30% vs. 6%) and almost identical between grade 1 and 2 intraepithelial lesions (22%) [66]. This is because the histological features of papillomavirus infection (namely, koilocytosis, multi-nucleation, acanthosis, papillomatosis, para-keratosis, individual cell keratinization, mitosis in the lower basal third of the epithelium, hyperplasia of the basal layers and absence of a layer of different basal cells) can be present in different ways, either localized or diffuse. This difference in proportion raises the question of the differential diagnosis between the changes associated with human papillomavirus infection and those relational or metaplastic [67]. The different proportions observed in the different studies may also be due to the different techniques used to identify these alterations.

4.6. PD-L1 and Tissue Alterations Predicting Papillomavirus Infection

The expression of PD-L1 distributed according to the different tissue alterations predictive of papillomavirus infection is variable in this study and is more found in lesions of the intraepithelial capillary type and basal hyperplasia, ie 37.5 and 25% respectively. Many studies with PD-L1 have shown that its expression is high in areas with T cell activity [68–71]. Indeed, the expression of PD-L1 is stronger at the level of the basal layer and of the intraepithelial capillaries due to the presence of T lymphocyte found respectively in the chorion and in the vascular lumen, thus carrying out the phenomenon of emperipoiesis [69]. Epithelial cells resistant to this anti-tumor immune mechanism develop PD-L1 on their membrane which binds to lymphocyte PD1 thus generating an immune tolerance system responsible for the progression from intraepithelial neoplasia to cervical cancer [95].

4.7. PD-L1 and Grades of Intraepithelial Neoplasms of the Cervix

PD-L1 expression was observed in 81.25% of grade 3 cervical intraepithelial neoplasms in this study. Intraepithelial neoplasms and its correlation with the persistence of human papillomavirus infection, expression of PD-L1 in cervical epithelial and mononuclear cells gradually increases from the CIN 1 group without Papillomavirus positivity to at oncogenic risk towards the oncogenic group CIN II-III positive for high-risk oncogenic papillomavirus with a significant difference between the respective groups ($p=0.0003$ and $p=0.0394$) [95]. By evaluating the capacity for recurrence of neoplastic intraepithelial lesions of the cervix after conization by looking for the expression of TPX2 and PD-L1 in cells of the surgical margin, the authors noted that the positive PD-L1 level was significantly higher in the CIN2-3 group (57.8%) than in the CIN1 / Normal group (29.4%) ($p=0.032$) [83]. These observations show that PD-L1 is a biomarker of intraepithelial neoplasia of ranking officer.

5. Conclusion

During this study, the various cytological and tissue alterations predictive of papillomavirus infection (koilocytosis, binucleation, dyskeratosis papillomatosis, acanthosis, intra-epithelial capillaries, Hyperplasia of the basal layer) were identified in intraepithelial neoplasia soft cervix. All the histological grade of intra-epithelial cervical neoplasia were identified. The expression of the PD-L1 biomarker of resistance to anti-tumor immunity is not present in all these alterations, but it remains more observed in intraepithelial neoplasias comprising alterations like the binucleation, the intraepithelial capillary and the basal layer hyperplasia as well as high grade ones. It is therefore desirable to consider further studies on the expression of several immune histochemical biomarkers of papillomavirus infection and HPV genotyping in relation to the morphological alterations induced by this virus in

intraepithelial neoplasms of the cervix.

References

- [1] Malagón T, Volesky KD, Bouten S, Laprise C, El-Zein M, Franco EL. Cumulative risk of cervical intraepithelial neoplasia for women with normal cytology but positive for human papillomavirus: systematic review and meta-analysis. *Int J Cancer*. 2020.
- [2] Riccio DA, Maeda-Chubachi T, Messersmith E, Geer C. Nitric oxide as a topical treatment for cervical intraepithelial neoplasia caused by high-risk human papillomavirus infection. *Med Res Arch*. 2020; 8 (6).
- [3] Escobar N, Plugge E. Prevalence of human papillomavirus infection, cervical intraepithelial neoplasia and cervical cancer in imprisoned women worldwide: a systematic review and meta-analysis. *J Epidemiol Community Health*. 2020; 74 (1): 95–102.
- [4] Gupta SM, Mania-Pramanik J. Molecular mechanisms in progression of HPV-associated cervical carcinogenesis. *J Biomed Sci*. 2019; 26 (1): 28.
- [5] von Knebel Doeberitz M, Cubie H, Broker TR, Jenkins D. Linking Human Papillomavirus to Human Cancer and Understanding Its Carcinogenic Mechanisms. In: *Human Papillomavirus*. Elsevier; 2020. p. 17–39.
- [6] He C, Lv X, Huang C, Angeletti PC, Hua G, Dong J, et al. A human papillomavirus-independent cervical cancer animal model reveals unconventional mechanisms of cervical carcinogenesis. *Cell Rep*. 2019; 26 (10): 2636–2650.
- [7] Vonsky M, Shabaeva M, Runov A, Lebedeva N, Chowdhury S, Palefsky JM, et al. Carcinogenesis associated with human papillomavirus infection. Mechanisms and potential for immunotherapy. *Biochem Mosc*. 2019; 84 (7): 782–799.
- [8] Chen L, Sun M, Shi H, He Q, Liu D. Association of human papillomavirus L1 capsid protein with koilocytosis, expression of p16, and Ki-67, and its potential as a prognostic marker for cervical intraepithelial neoplasia. *Anal Quant Cytopathol Histopathol*. 2013; 35 (3): 139–145.
- [9] Yoon HJ, Jeong M, Ki EY, Lee SJ, Lee YS. EP345 Human papillomavirus L1 capsid protein and HPV test as a biomarker for cervical intraepithelial neoplasia 2+ in women with persistent ASCUS/LSIL cervical cytology. *BMJ Specialist Journals*; 2019.
- [10] Seresht LM. EP368 Prognostic value of KI6 biomarker in predict short time prognosis of low grade cervical intraepithelial neoplasia in HPV negative and positive patient. *BMJ Specialist Journals*; 2019.
- [11] Yang F, Cui Z, Liao Y, Tian R, Fan W, Jin Z, et al. FHIT and C-MYC expression in cervical histology and cytology as biomarkers for detecting high-grade intraepithelial neoplasia in human papillomavirus-positive women. *Cancer Biomark*. 2020; (Preprint): 1–10.
- [12] Ara R, Pervin S, Khatun S, Shahera U, Sultana M, Aktar MN. Low-Cost Molecular Biomarker HPV-16/18 E6 Oncoprotein Expression in Cervical Intraepithelial Neoplasia (CIN) and Cervical Cancer with Its Relation with Severity of Neoplastic State. *Indian J Gynecol Oncol*. 2020; 18 (3): 1–5.
- [13] Didier M, Veronique K, Abdon M, Prosper K, Julien I. Cervical Intra-Epithelial Neoplasia: Index of Papillomavirus Infection and Histopathological Grade in Lubumbashi Patients. *Int J Clin Oncol Cancer Res*. 2019; 4 (3): 29.
- [14] Amaro-Filho SM, Gradissimo A, Usyk M, Moreira FC, de Almeida LM, Moreira MA, et al. HPV73 a nonvaccine type causes cervical cancer. *Int J Cancer*. 2020; 146 (3): 731–738.
- [15] Carcopino X. Citología cervical y conducta práctica en caso de frotis anormal. *EMC-Ginecol-Obstet*. 2019; 55 (1): 1–11.
- [16] Pereira-Caldeira NMV, Góes FGB, de Almeida-Cruz MCM, de Souza Caliani J, Pereira-Ávila FMV, Gir E. Quality of Life for Women with Human Papillomavirus-induced Lesions. *Rev Bras Ginecol E ObstetríciaRBGO Gynecol Obstet*. 2020; 42 (04): 211–217.
- [17] Wu SC, Canarte V, Beeravolu H, Grace M, Sharma S, Munger K. Finding How Human Papillomaviruses Alter the Biochemistry and Identity of Infected Epithelial Cells. In: *Human Papillomavirus*. Elsevier; 2020. p. 53–65.
- [18] Salim RC, Lima TM, Heinke T, Prigenzi KC, Speck NMG, Focchi GR. Cervical squamous intraepithelial lesions grades 1 and 2: value of an alternative morphological classification and of p16 immunohistochemistry in the prediction of clinical outcome. *J Bras Patol E Med Lab*. 2017; 53 (5): 313–319.
- [19] GOULART APS, GONÇALVES MAG, DA-SILVA VD. Evaluation of Telomerase (hTert), Ki67 and p16ink4a expressions in low and high-grade cervical intraepithelial lesions. *Rev Colégio Bras Cir*. 2017; 44 (2): 131–139.
- [20] de Melo FLP, Lancellotti CLP, da Silva MALG. Expression of the immunohistochemical markers p16 and ki-67 and their usefulness in the diagnosis of cervical intraepithelial neoplasms. *Rev Bras Ginecol E ObstetríciaRBGO Gynecol Obstet*. 2016; 38 (02): 082–087.
- [21] Han C, Zhao F, Wan C, He Y, Chen Y. Associations between the expression of SCCA, MTA1, P16, Ki-67 and the infection of high-risk HPV in cervical lesions. *Oncol Lett*. 2020; 20 (1): 884–892.
- [22] WANG H, JIN Y, NI C, TIAN X, WANG W, ZHAO Y. Correlation between ER, PR, P53, Ki67 Expression and High-Risk HPV Infection in Patients with Different Levels of Cervical Intraepithelial Neoplasia. *Indian J Pharm Sci*. 2020; 82 (3): 1–7.
- [23] Kawashita S, Matsuda K, Kondo H, Kitajima Y, Hasegawa Y, Shimada T, et al. Significance of p53-Binding Protein 1 Nuclear Foci in Cervical Squamous Intraepithelial Lesions: Association With High-Risk Human Papillomavirus Infection and P16INK4a Expression. *Cancer Control*. 2020; 27 (1): 1073274819901170.
- [24] Zhao J, Zhu J, Guo J, Zhu T, Zhong J, Liu M, et al. Genetic variability and functional implication of HPV16 from cervical intraepithelial neoplasia in Shanghai women. *J Med Virol*. 2020; 92 (3): 372–381.
- [25] Vogelsang TL, Schmoeckel E, Kuhn C, Blankenstein T, Temelkov M, Heidegger H, et al. Regulation of LCoR and RIP140 expression in cervical intraepithelial neoplasia and correlation with CIN progression and dedifferentiation. *J Cancer Res Clin Oncol*. 2020; 1–9.
- [26] Baba S, Taguchi A, Kawata A, Hara K, Eguchi S, Mori M, et al. Differential expression of human papillomavirus 16-, 18-, 52-, and 58-derived transcripts in cervical intraepithelial neoplasia. *Virol J*. 2020; 17 (1): 1–10.

- [27] Farghaly SA. Immunotherapy for Precancerous Lesions of the Uterine Cervix. In: *Uterine Cervical Cancer*. Springer; 2019. p. 107–140.
- [28] Wakabayashi R, Nakahama Y, Nguyen V, Espinoza JL. The host-microbe interplay in human papillomavirus-induced carcinogenesis. *Microorganisms*. 2019; 7 (7): 199.
- [29] Kerr PS, Freedland SJ, Williams SB. The current status of molecular biomarkers in patients with metastatic urothelial carcinoma of the bladder. Taylor & Francis; 2020.
- [30] Evans ET, Castellano T, Brown AS, Dvorak J, Henson CE, Gunderson CC. The Effect of BMI on Radiation Dosing And Survival In Patients With Locally Advanced Cervical Cancer. *Gynecol Oncol*. 2020; 156 (3): e9–e10.
- [31] Marcq* G, Souhami L, Cury F, Aprikian A, Tanguay S, Vanhuyse M, et al. MP61-08 A PHASE I/II TRIAL OF TRANSURETHRAL SURGERY FOLLOWED BY A COMBINATION OF ATEZOLIZUMAB AN ANTI-PDL-1 (MPDL3280A) WITH TRIMODAL THERAPY IN PATIENTS WITH MUSCLE-INVASIVE BLADDER CANCER. *J Urol*. 2020; 203 (Supplement 4): e938–e938.
- [32] Walankiewicz M, Grywalska E, Korona-Glowniak I, Rolinski J, Kotarski J. Alteration of the PD-1/PD-L1 axis in cervical intraepithelial neoplasia-preliminary study. *Eur J Obstet Gynecol Reprod Biol*. 2019; 234: e76.
- [33] Mezache L, Paniccia B, Nyinawabera A, Nuovo GJ. Enhanced expression of PD L1 in cervical intraepithelial neoplasia and cervical cancers. *Mod Pathol*. 2015; 28 (12): 1594–1602.
- [34] Yang W, Lu Y-P, Yang Y-Z, Kang J-R, Jin Y-D, Wang H-W. Expressions of programmed death (PD)-1 and PD-1 ligand (PD-L1) in cervical intraepithelial neoplasia and cervical squamous cell carcinomas are of prognostic value and associated with human papillomavirus status. *J Obstet Gynaecol Res*. 2017; 43 (10): 1602–1612.
- [35] Chinn Z, Stoler MH, Mills AM. PD-L1 and IDO expression in cervical and vulvar invasive and intraepithelial squamous neoplasias: implications for combination immunotherapy. *Histopathology*. 2019; 74 (2): 256–268.
- [36] Fimiani M, Mazzatenta C, Biagioli M, Andreassi L. Vulvar squamous papillomatosis and human papillomavirus infection. A polymerase chain reaction study. *Arch Dermatol Res*. 1993; 285 (5): 250–254.
- [37] Gillison ML, Alemany L, Snijders PJ, Chaturvedi A, Steinberg BM, Schwartz S, et al. Human papillomavirus and diseases of the upper airway: head and neck cancer and respiratory papillomatosis. *Vaccine*. 2012; 30: F34–F54.
- [38] Didier M, Véronique K, Philippe M, Abdon M, Joseph C, Prosper K, et al. Human Papillomavirus and Cervical Intra-Epithelial Neoplasia: Epidemiological and Cytological Study in Lubumbashi Women. *Int J Clin Oncol Cancer Res*. 2019; 4 (1): 1.
- [39] Cao L, Sun P-L, Yao M, Chen S, Gao H. Clinical significance of CK7, HPV-L1, and koilocytosis for patients with cervical low-grade squamous intraepithelial lesions: a retrospective analysis. *Hum Pathol*. 2017; 65: 194–200.
- [40] Siebers AG, van der Linden H, Vedder JEM, Bekkers RLM, Melchers WLG, Bulten J. Presence of koilocytosis in low-grade smears of high-risk HPV-positive women is a negative predictor for cervical intraepithelial neoplasia grade 3 or more. *Cytopathology*. 2018; 29 (3): 275–280.
- [41] Park KJ. Cervical adenocarcinoma: integration of HPV status, pattern of invasion, morphology and molecular markers into classification. *Histopathology*. 2020; 76 (1): 112–127.
- [42] Vrdoljak-Mozetič D, Krašević M, Verša Ostojić D, Štemberger-Papić S, Rubeša-Mihaljević R, Bubonja-Šonje M. HPV 16 genotype, p16/Ki-67 dual staining and koilocytic morphology as potential predictors of the clinical outcome for cervical low-grade squamous intraepithelial lesions. *Cytopathology*. 2015; 26 (1): 10–18.
- [43] Gayatree A, Tanveer N, Arora VK, Arora V. Are Histomorphological Features Predictive of p16 Immunopositivity Different for Oral and Oropharyngeal Squamous Cell Carcinoma? *Indian J Surg Oncol*. 2020; 11 (2): 248–255.
- [44] Falcón MF, Paradedá ME, Kamermann FG, Maldonado V, Díaz L, Cardinal L. Immunohistochemistry of p16 and p53 in vulvar cancer. *Medicina (Mex)*. 2020; 80 (2): 127–133.
- [45] Katumabayi JC, et al. Intraepithelial Neoplasias of uterine cervix in the Congolese in Kinshasa: interest of immunohistochemical biomarker. *Ann africaines de medecine*; Mai-2020
- [46] Krause KA, Butler SL. Koilocytosis. In: *StatPearls* [Internet]. StatPearls Publishing; 2018.
- [47] Eble JN, Tavassoli FA, Devilee P. Pathology and Genetics of Tumours of the Breast and Female Genital Organs. *Iarc*; 2003.
- [48] Tavassoli FA. Pathology and genetics of tumours of the breast and female genital organs. *World Health Organ Classif Tumours*. 2003.
- [49] Smith J, Robida MD, Acosta K, Vennapusa B, Mistry A, Martin G, et al. Quantitative and qualitative characterization of two PD-L1 clones: SP263 and E1L3N. *Diagn Pathol*. 2016; 11 (1): 1–9.
- [50] Silva NNT, Sabino A de P, Tafuri A, Lima AA. Lack of association between methylenetetrahydrofolate reductase C677T polymorphism, HPV infection and cervical intraepithelial neoplasia in Brazilian women. *BMC Med Genet*. 2019 Jun 6; 20 (1): 100.
- [51] Didier M, Phillipe M, Abdon M, Julien I. Cervical smears: morphological and epidemiological study for intra-epithelial neoplasia in Lubumbashi. *Int J Clin Oncol Cancer Res*. 2018; 3 (4): 55–58.
- [52] Attiya AM, Ali AE, AbdelSalam HS, Abdelwahab MM. Frequency of Cervical Intra-epithelial Neoplasia in Oral Contraceptive Pills Users in Zagazig University Hospitals. *Zagazig Univ Med J*. 2020 Jul 1; 26 (4): 590–7.
- [53] Paluku JL, Carter TE, Lee M, Bartels SA. Massive single visit cervical pre-cancer and cancer screening in eastern Democratic Republic of Congo. *BMC Womens Health*. 2019 Mar 4; 19 (1): 43.
- [54] Utoo. Cervical intraepithelial neoplasia: Prevalence, risk factors, and utilization of screening services among an urban population in Nigeria [Internet]. [cited 2020 Jul 21]. Available from: <http://www.tjgonline.com/article.asp?issn=0189-5117; year=2016; volume=33; issue=3; page=279; epage=283; aulast=Utoo>.

- [55] Sanad AS, Kamel HH, Hasan MM. Prevalence of cervical intraepithelial neoplasia (CIN) in patients attending Minia Maternity University Hospital. *Arch Gynecol Obstet.* 2014 Jun; 289 (6): 1211–7.
- [56] Adekunle OO. Prevalence of cervical intraepithelial neoplasia in Zaria. *Ann Afr Med.* 2010; 9 (3).
- [57] Ceccato Junior BPV, Guimarães MDC, Lopes APC, Nascimento LF, Novaes LM, Castillo DM del, et al. Incidence of Cervical Human Papillomavirus and Cervical Intraepithelial Neoplasia in Women with Positive and Negative HIV Status. *Rev Bras Ginecol E Obstet.* 2016 May; 38 (5): 231–8.
- [58] Wang X, Zeng Y, Huang X, Zhang Y. Prevalence and Genotype Distribution of Human Papillomavirus in Invasive Cervical Cancer, Cervical Intraepithelial Neoplasia, and Asymptomatic Women in Southeast China [Internet]. Vol. 2018, *BioMed Research International.* Hindawi; 2018 [cited 2020 Jul 21]. p. e2897937. Available from: <https://www.hindawi.com/journals/bmri/2018/2897937/>.
- [59] Kelly H, Weiss HA, Benavente Y, de Sanjose S, Mayaud P, Qiao Y, et al. Association of antiretroviral therapy with high-risk human papillomavirus, cervical intraepithelial neoplasia, and invasive cervical cancer in women living with HIV: a systematic review and meta-analysis. *Lancet HIV.* 2018 Jan 1; 5 (1): e45–58.
- [60] Zhao S, Zhao X, Hu S, Lu J, Duan X, Zhang X, et al. Distribution of high-risk human papillomavirus genotype prevalence and attribution to cervical precancerous lesions in rural North China. *Chin J Cancer Res.* 2019 Aug; 31 (4): 663–72.
- [61] Ma L, Bian M-L, Cheng J-Y, Xiao W, Hao M, Zhu J, et al. Hybrid capture II for high-risk human papillomavirus DNA testing to detect cervical precancerous lesions: A qualitative and quantitative study. *Exp Ther Med.* 2010 Jan 1; 1 (1): 193–8.
- [62] Na K, Sung J-Y, Kim H-S. Clinicopathological Characteristics of High-grade Squamous Intraepithelial Lesions Involving Condyloma Acuminatum. *Anticancer Res.* 2018 Jan 3; 38 (3): 1767–74.
- [63] Missaoui N, Trabelsi A, Hmissa S, Fontanière B, Yacoubi MT, Mokni M, et al. p16INK4A overexpression in precancerous and cancerous lesions of the uterine cervix in Tunisian women. *Pathol-Res Pract.* 2010; 206 (8): 550–555.
- [64] Mandic A, UsajKnezevic S, KapićIvkovic T. Tissue expression of VEGF in cervical intraepithelial neoplasia and cervical cancer. *J BUON.* 2014; 19 (4): 958–64.
- [65] No JH, Jo H, Kim S-H, Park I-A, Kang D, Lee CH, et al. Expression of MMP-2, MMP-9, and urokinase-type plasminogen activator in cervical intraepithelial neoplasia. *Ann N Y Acad Sci.* 2009; 1171 (1): 100.
- [66] Guillaud M, Buys TPH, Carraro A, Korbelik J, Follen M, Scheurer M, et al. Evaluation of HPV Infection and Smoking Status Impacts on Cell Proliferation in Epithelial Layers of Cervical Neoplasia. *PLOS ONE.* 2014 Sep 11; 9 (9): e107088.
- [67] Cabibi D, Giovannelli L, Martorana A, Migliore MC, Tripodo C, Campione M, et al. Predictive role of histological features and Ki67 pattern on high-risk HPV presence in atypical cervical lesions. 2007.
- [68] Roux C. Activité immunosuppressive des cellules stromales mésenchymateuses dérivées de cellules souches pluripotentes induites humaines: induction de lymphocytes T régulateurs in vitro et in vivo et expression de PD-L1 [PhD Thesis]. 2018.
- [69] Vassilakopoulou M, Avgeris M, Velcheti V, Kotoula V, Rampias T, Chatzopoulos K, et al. Evaluation of PD-L1 expression and associated tumor-infiltrating lymphocytes in laryngeal squamous cell carcinoma. *Clin Cancer Res.* 2016; 22 (3): 704–713.
- [70] Beyrend G, van der Gracht E, Yilmaz A, van Duikeren S, Camps M, Höllt T, et al. PD-L1 blockade engages tumor-infiltrating lymphocytes to co-express targetable activating and inhibitory receptors. *J Immunother Cancer.* 2019; 7 (1): 1–14.
- [71] Tsang JY, Au W-L, Lo K-Y, Ni Y-B, Hlaing T, Hu J, et al. PD-L1 expression and tumor infiltrating PD-1+ lymphocytes associated with outcome in HER2+ breast cancer patients. *Breast Cancer Res Treat.* 2017; 162 (1): 19–30.
- [72] Winkler B, Crum CP, Fujii T, Ferenczy A, Boon M, Braun L, et al. Koilocytotic lesions of the cervix. The relationship of mitotic abnormalities to the presence of papillomavirus antigens and nuclear dna content. *Cancer.* 1984; 53 (5): 1081–7.
- [73] Etchebehere RM, Almeida ÉCS, Côbo EC, Duque AC da R, Murta EFC, Adad SJ, et al. Comparison of Classical and Secondary Cytologic Criteria Relative to Hybrid Capture for Diagnosing Cervical-vaginal Infection by Human Papillomavirus. *Rev Bras Ginecol E Obstet.* 2016 Jan; 38 (1): 41–6.
- [74] Washiya K, Motoi M, Kobayashi T, Yoshioka H, Watanabe J. Significance of binucleated cells with compression in atypical squamous cells of undetermined significance. *Acta Cytol.* 2013; 57 (6): 599–603.
- [75] Win KP, Kitjaidure Y, Hamamoto K, Myo Aung T. Computer-Assisted Screening for Cervical Cancer Using Digital Image Processing of Pap Smear Images. *Appl Sci.* 2020 Jan; 10 (5): 1800.
- [76] Meziani S, Haoud K, Mehida H, Menadi N, Chenni FZ, Bekhaled I, et al. Epidemiological Approach and Precocious Diagnosis of Precancerous Cervical Lesion in Sidi Bel Abbes Region (North-West of Algeria). *J Drug Deliv Ther.* 2020; 10 (1-s): 72–78.
- [77] Kir G, Sarbay BC, Seneldir H. The significance of parakeratosis alone in cervicovaginal cytology of turkish women. *Diagn Cytopathol.* 2017; 45 (4): 297–302.
- [78] Bedin R, Gasparin VA, de Brito Pitilin É. Fatores associados às alterações cérvico-uterinas de mulheres atendidas em um município polo do oeste catarinense Factors associated to uterine-cervix changes in women assisted in a pole town in western Santa Catarina. *Rev Pesqui Cuid É Fundam Online.* 2017; 9 (1): 167–174.
- [79] Shetty R, Hebbar A, Kulkarni N. Pattern of Cervical Cytology using Papanicolaou Stain: An Experience from a Tertiary Hospital. *Call Editor Board Memb.* 2020; 13 (1): 83.
- [80] Muntean M. Cytological identification of the (pre) cancerous cervical lesions within a clinically asymptomatic female population. *Curr Health Sci J.* 2009; 35: 176–179.

- [81] Hong SR, Kim BM, Kim HS, Chun YK, Kim HS. Evaluation of Low-Grade Squamous Intraepithelial Lesions, Cannot Exclude High-Grade Squamous Intraepithelial Lesions on Cervical Smear. *Korean J Pathol.* 2010; 44 (5): 528.
- [82] Hunter C, Duggan MA, Duan Q, Power P, Gregoire J, Nation J. Cytology and outcome of LSIL: cannot exclude HSIL compared to ASC-H. *Cytopathology.* 2009; 20 (1): 17–26.
- [83] Zhang H, Zhang T, You Z, Zhang Y. Positive Surgical Margin, HPV Persistence, and Expression of Both TPX2 and PD-L1 Are Associated with Persistence/Recurrence of Cervical Intraepithelial Neoplasia after Cervical Conization. *PLOS ONE.* 2015 Dec 1; 10 (12): e0142868.
- [84] Curley J, Conaway MR, Chinn Z, Duska L, Stoler M, Mills AM. Looking past PD-L1: expression of immune checkpoint TIM-3 and its ligand galectin-9 in cervical and vulvar squamous neoplasia. *Mod Pathol.* 2020 Jun; 33 (6): 1182–92.
- [85] Liu C, Lu J, Tian H, Du W, Zhao L, Feng J, et al. Increased expression of PD-L1 by the human papillomavirus 16 E7 oncoprotein inhibits anticancer immunity. *Mol Med Rep.* 2017 Mar 1; 15 (3): 1063–70.
- [86] Nuovo G, Schwartz Z, Magro C. A comparison of the detection of biomarkers in infections due to low risk versus high-risk human papillomavirus types. *Ann Diagn Pathol.* 2019 Aug 1; 41: 57–61.
- [87] Khalbuss WE, Pantanowitz L, Monaco SE. Cytomorphology of unusual primary tumors in the Pap test. *Cytojournal.* 2013; 10.
- [88] Stoler MH, Jenkins D, Bergeron C. The Pathology of Cervical Precancer and Cancer and its importance in clinical practice. In: *Human Papillomavirus.* Elsevier; 2020. p. 85–109.
- [89] Léonard B, Kridelka F, Delbecq K, Goffin F, Demoulin S, Doyen J, et al. A clinical and pathological overview of vulvar condyloma acuminatum, intraepithelial neoplasia, and squamous cell carcinoma. *BioMed Res Int.* 2014; 2014.
- [90] de Oliveira GG, Eleutério RMN, Barbosa R de CC, de Almeida PRC, Eleutério Jr J. Atypical squamous cells: cytopathological findings and correlation with HPV genotype and histopathology. *Acta Cytol.* 2018; 62 (5–6): 386–392.
- [91] Leonardo E, Bardales RH. Exfoliative Cytology. In: Leonardo E, Bardales RH, editors. *Practical Immunocytochemistry in Diagnostic Cytology* [Internet]. Cham: Springer International Publishing; 2020 [cited 2020 Aug 7]. p. 41–67. Available from: https://doi.org/10.1007/978-3-030-46656-5_3.
- [92] Tambas M, Altun M, Tural D. Human papillomavirus in head and neck cancer. *Hum Papillomavirus Res Glob Perspect.* 2016; 255.
- [93] Foy J-P, Bertolus C, Boutolleau D, Agut H, Gessain A, Herceg Z, et al. Arguments to Support a Viral Origin of Oral Squamous Cell Carcinoma in Non-Smoker and Non-Drinker Patients. *Front Oncol.* 2020; 10: 822.
- [94] Tracht J, Robinson BS, Krasinskas AM. Pathology of Premalignant and Malignant Disease of the Esophagus. In: *Esophageal Cancer.* Springer; 2020. p. 61–81.
- [95] Sancakli Usta C, Altun E, Afsar S, Bulbul CB, Usta A, Adalı E. Overexpression of programmed cell death ligand 1 in patients with CIN and its correlation with human papillomavirus infection and CIN persistence. *Infect Agent Cancer.* 2020 Jul 17; 15 (1): 47.